

**PHYSICO-CHEMICAL CHARACTERISTICS, SENSORY PROFILE AND SHELF
STABILITY OF BREAD INCORPORATING SHELF-STORABLE ORANGE FLESHED
SWEETPOTATO PUREE**

BY

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2018

DECLARATION

I, **OMBAKA JOSHUA OWADE**, hereby declare that this dissertation is my original work and has not been submitted for a degree in any other institution.

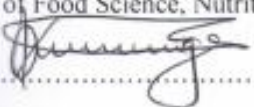
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TURNITIN ORIGINALITY REPORT

DEDICATION

This work is dedicated unto you, the late sister Florence Anyango Owade and all who love and care for me and have supported me this far, and so we say:

To God be the glory, Great things He hath done

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I glorify the Almighty God who has been steadfast upon His mercy, grace and favour. *“Surely goodness and mercy shall follow me...”*

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LIST OF ACRONYMNS

ANOVA	Analysis of variance
AOAC	Association of Analytical Chemists
CIP	International Potato Centre
cm	centimeters
FAO	Food and Agriculture Organization
g	Grams
HPLC	High Performance Liquid Chromatography
ILRI	International Livestock Research Institute
Kg	Kilograms
log cfu	Logarithmic colony forming unit
LSD	Least Significant Difference
ml	Milliliters
OFSP	Orange-Fleshed Sweetpotato
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
RAE	Retinol Activity Equivalents
RH	Relative humidity
SSA	Sub-Saharan Africa

TVC Total Viable Count

USFDA United States Food and Drug Administration

VAD Vitamin A Deficiency

OPERATIONAL DEFINITION OF TERMS

Fresh puree: Orange-fleshed sweetpotato puree that has not been preserved using vacuum packaging technology and thereby subjected to the cold storage.

Keeping quality: It is the length or period of storage that the product remains palatable under certain conditions.

Preservative: A substance or chemical that is added to food to prevent food spoilage and decomposition due to microbial action or undesirable chemical changes.

Shelf-storable puree: Orange-fleshed sweetpotato puree that has been preserved with citric acid, sodium benzoate and potassium sorbate and vacuum packaging and is usually stored in shelf.

GENERAL ABSTRACT

Orange fleshed sweet potato (OFSP) puree has been promoted as a functional ingredient in bread with the overall aim of alleviating vitamin A deficiency (VAD). OFSP puree bread has been commercialized in many sub-Saharan Africa countries with over-reliance on the cold storage in its supply. However, this has resulted into higher costs to producers as it requires additional capital and lacks stability in the supply of the puree for production. The current study was designed with the overall objective of evaluating the use of shelf-storable OFSP puree as an alternative to the fresh OFSP puree in bread production. The study employed an experimental study design with factorial arrangement where two factors including different treatments of shelf-storable OFSP puree and periods of storage. OFSP puree sample was dosed with different combinations of chemical preservatives: treatment 1 with 0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid and treatment 2 with 0.2% potassium sorbate + 0.2% sodium benzoate + 1% citric acid. Each of the treated puree was stored at ambient conditions for a period of four months. The OFSP purees were sampled monthly for analysis and incorporation into bread at 30% and 40%. Bread in which fresh OFSP puree incorporated at similar levels and wheat breads acted as controls. The nutritional composition of puree and bread, physical attributes and microbial stability of the breads were determined.

The results showed 45.6% and 57.3% reduction in β -carotene content in both treatment 1 and 2 of shelf-storable OFSP purees respectively by the fourth month ($p < 0.05$). Bread samples made by incorporating 40% shelf-storable OFSP puree provided significant ($p < 0.05$) levels of β -carotene to consumers up to three months of storage; treatment 2 shelf-storable OFSP bread provided up to 121.30 ± 8.05 RAE. The crude ash and moisture contents of shelf-storable OFSP

puree bread were significantly ($p < 0.05$) higher than that of the control wheat bread. The proximate composition of bread made by incorporating puree sampled at different months did not differ significantly ($p > 0.05$). The loaf weight, volume and specific volume of bread from shelf-storable and fresh OFSP purees as well as wheat bread were not significantly different ($p > 0.05$).

The most acceptable breads was one in which 40% fresh OFSP puree was incorporated ($p < 0.05$). The two treatments did not differ significantly ($p > 0.05$) in acceptability of the breads when compared to wheat bread. The saltiness, smoothness, crumb colour and crust colour of shelf-storable OFSP puree bread was significantly higher ($p < 0.05$) than the wheat bread but similar ($p > 0.05$) to fresh puree bread. Microbial tests revealed that incorporation of OFSP puree, whether fresh or shelf-storable, into bread resulted into lower yeast and mold counts ($p < 0.05$). Aerobic counts in shelf-storable OFSP puree bread increased with the period of storage of the OFSP puree ($p < 0.05$). Shelf-storable OFSP puree bread could be stored for seven days with no visible yeast and mould spoilage. The current study found that shelf-storable OFSP puree, notwithstanding the level of preservatives, can be an alternative to fresh puree in the substitution of wheat flour with OFSP puree in bread production. Shelf-storable OFSP puree can therefore be promoted as an alternative to fresh OFSP puree in bread production.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 BACKGROUND INFORMATION

Sweetpotato, *Ipoemea batatas* L. belongs to the class Dicotyledonae and the Family Convovulaceae (Koala *et al.*, 2013). The genus *Ipoemae* is estimated to have more than 400 species, many of which are wild (Dooshima, 2014). The global sweet potato production in 2014 was estimated to be over 106 million tonnes out of which the Kenyan production was estimated to be 763,643 tonnes (FAOSTAT, 2017). Sweetpotato varieties or cultivars have been developed to suit different needs and utilization, with varied colours (white, cream, yellow, orange and purple) and varied dry matter and starch contents and varied sugar contents (sweet and non-sweet varieties) (Wheatley and Loechl, 2008). In as much as sweet potato has high carbohydrate content, it has a low glycemic index indicating a low carbohydrate digestibility (Fetuga *et al.*, 2014). The leaves and tubers are good sources of antioxidants, fiber, vitamin C and pro-vitamin A and some mineral elements such as zinc, potassium, sodium, manganese, calcium, magnesium and iron (Burri, 2011).

Orange fleshed sweetpotato (OFSP) has been used to combat vitamin A deficiency with great success in many countries (Koala *et al.*, 2013; Jenkins *et al.*, 2015). It has been noted that sweetpotato is still underutilized and there is need to expand the utilization and market opportunities for sweet potato including the production of products which are adapted to consumer preferences (Fetuga *et al.*, 2014). Several products ,as shown by various documented cases, made from OFSP are noted to have high consumer preferences. They include OFSP flour

(Ukpabi, 2012), OFSP chips (Fetuga *et al.*, 2014), OFSP juice (Mamo *et al.*, 2014), OFSP crisps and OFSP bread (Andrade *et al.*, 2016).

A large proportion of the industrial production of bread in Kenya and sub-Saharan Africa (SSA) relies on wheat as the raw material which is largely imported, and thus involve huge expenditure of foreign exchange leading to high cost of bread which may be unaffordable to a large proportion of the consumer population (Dooshima, 2014). This creates the need for a cheaper and more reliable option such as the use of OFSP. OFSP has been used in bread production as puree and flour with the puree based bread recording a higher consumer acceptability (Muzhingi, 2016). The OFSP puree based bread was noted to have a higher acceptance when compared to OFSP flour based bread and wheat wheat bread (Bonsi *et al.*, 2014). In SSA, the use of OFSP puree as a substitute of wheat flour in the production of bread is a more economically viable venture compared to OFSP flour due to the cost of the latter in the region (Bocher *et al.*, 2016). OFSP puree that has been subjected to cold storage is currently in use in bread production, however, it presents challenges in terms of additional capital needed and instability in OFSP puree supply. Advances in research have enabled OFSP puree to be preserved through vacuum packaging to lengthen its shelf-life and preserve its quality factors. The storage of OFSP puree by preserving it with a combination of potassium sorbate and sodium benzoate and then vacuum packaging leads to retention of high beta carotene content (Daniel and Magnaghi, 2016). However the effect of this mode of preservation on the sensory quality and stability of the bread made from the shelf-stable puree is not known. The current study seeks to establish the physico-chemical attribute, sensory quality and shelf-life of bread made from the shelf-stable OFSP puree.

1.2 PROBLEM STATEMENT

Consumption of OFSP based bread in Kenya has been on the increase as consumers continue to look for nutritious alternative foods. Incorporation of fresh OFSP puree into wheat for bread making is widely adopted in Kenya due to its cost effectiveness and nutritional superiority over OFSP flour. The fresh OFSP puree exploited in this has a high perishability especially from microbial contamination, thus has limited keeping ability. The current shortfall has been addressed through frozen storage of the puree. This, however, attracts additional costs thus expensive and massive inconsistency in puree supply is experienced. In order to expand the use of OFSP puree across the country and in all season while enhancing the cost-effectiveness, there is need for an alternative storage method. As a result, research into ways of puree preservation has resulted into production of a shelf-storable OFSP puree that can be stored for three to six months at prevalent weather conditions in Kenya with no significant quality changes. This puree is preserved using acceptable preservatives such as potassium sorbate, sodium benzoate and citric acid. However, the quality and sensory characteristics and shelf stability of bread prepared by substitution of wheat flour with shelf-storable OFSP puree has not been systematically evaluated in relation to that prepared from conventional fresh OFSP puree. The current research sought to establish whether bread made by substituting wheat flour with shelf-storable puree is significantly different from bread made from fresh puree.

1.3 JUSTIFICATION

Fresh OFSP puree has been used in substituting wheat for production of vitamin A rich bread which is commercially traded in Kenya (Bocher *et al.*, 2016). The fresh puree is, however, highly perishable and difficult to handle and hence requires electric power to freeze it as a means of

preservation. This practice increases the cost that has to be passed to the consumers. Adoption of a shelf-stable OFSP puree in bread making is envisaged to ease handling of the puree, reduce cost of bread making as well increase the availability of the puree throughout the year. This will eliminate seasonal variation of the availability of the puree given that sweetpotatoes are mainly produced using rainfed agriculture and hence mainly supplied in plenty about twice in a year. Similarly, adoption of shelf-storable OFSP puree will improve intake of raw sweetpotatoes during seasons of glut and thus stabilize prices leading to improved economic gain for both farmers and processors.

1.4 AIM

The aim of this study is to contribute towards reduction of vitamin A deficiency among the sub-Saharan Africa population.

1.5 PURPOSE

The purpose of this study is to provide information on the quality and sensory properties of bread made by substituting wheat flour with shelf-storable OFSP puree.

1.6 OBJECTIVES

1.6.1 Overall Objective

To determine the physico-chemical quality, sensory characteristics and shelf stability of bread prepared by substituting wheat flour with shelf-stable OFSP puree.

1.6.2 Specific Objectives

1. To determine the physico-chemical characteristics of bread made by substituting wheat flour with fresh and shelf-storable OFSP puree.
2. To determine the sensory profile of shelf-stable OFSP puree based bread in terms of colour, taste, flavor, texture and overall acceptability.
3. To determine the shelf stability of bread prepared by substituting wheat flour with shelf-storable OFSP puree.

1.7 HYPOTHESES

The overall hypothesis is that bread made by substituting wheat flour with shelf-storable OFSP puree is not significantly different from bread made from fresh OFSP puree and popular wheat bread. Hypothesis for each specific objective are as follows:

1. Bread made by substituting wheat flour with shelf-storable OFSP puree does not differ significantly in physico-chemical characteristics from that of fresh OFSP puree and conventional wheat bread.
2. Bread made by substituting wheat flour with shelf-storable OFSP puree has no significant differences in sensory acceptability from that of fresh OFSP puree and conventional wheat bread.
3. The keeping quality of bread made by substituting wheat flour with shelf-storable OFSP puree does not significantly differ from that of fresh OFSP puree and conventional wheat bread.

CHAPTER TWO: LITERATURE REVIEW

2.1 SUMMARY

Bread though an exotic food product in sub-Saharan Africa (SSA), has been an important cereal product consumed by most individuals among the vast sub-Saharan African population. Both in the local and industrial production, bread formulation has undergone evolution, with the latest and emerging technology being the incorporation of orange-fleshed sweetpotato (OFSP) puree in bread. OFSP puree-based bread is commercially available across SSA and is being promoted due to the potential nutritional benefits that it possesses. Together with OFSP flour based bread, OFSP puree based bread serves as a good food vehicle for β -carotene; this serves to alleviate vitamin A deficiency (VAD) especially among the vulnerable population in SSA. Using OFSP puree is better than using OFSP flour based on economic and nutritional considerations and hence the many initiatives promoting the production of OFSP puree based bread. The production of OFSP puree based bread has so far been relying on fresh OFSP puree or cold-chain stored OFSP puree. However, this has presented economic challenges and problems to the sustainability and expansion in production. With the development of shelf-storable preservative treated OFSP puree, most of these challenges will be overcome without undoing the currently harnessed benefits. The use of OFSP puree in bread baking can then be expanded easily at minimal production costs and maximum retention of nutritional quality. However, the use of the shelf-storable OFSP puree in bread baking needs to be evaluated further to present a substantiated case for its use. Great scientific interest in OFSP puree based bread production exists, but these advancements are only documented in scattered literature. The current review has been developed with focus on the scientific advances in the production of OFSP puree based bread

from both a historical and a forecast perspective. The scientific progress and breakthroughs in the use of OFSP puree in bread are critically reviewed.

2.2 INTRODUCTION

Bread is a baked product of flour or meal of cereals, especially wheat and includes ordinary, unleavened and leavened types (FAO, 1994). Bread production dates back to over 12,000 years ago (around 10000 BC) with the Egyptians as the pioneers, and was probably a deliberate experimentation with water and grain flour (Mondal and Datta, 2008). Bread is a widely consumed breakfast cereal globally with a quite diverse recipe (Williams, 2014). Baking of leavened bread must have been developed accidentally through the exposure of crushed grain to yeast cultures. It is estimated that around 1,000 BC, the Egyptians isolated yeast and used it in bread baking helping them to produce bread to the tune of thirty varieties (Salem *et al.*, 2015). The spontaneous fermentation of bread was replaced with controlled process of fermentation in late 19th Century yielding increased fermentation speed and better bread quality and consistency (Aslankoochi *et al.*, 2016).

Bread production has spread all over the world with different countries having different kinds of domesticated bread production methods (Gori *et al.*, 2010). Bread production has evolved over the ages in terms of ingredients used. Latest developments in bread production has led to the advancements in the bread industry that have enabled the use of various composite flours including purees to produce bread for improvement in the sensory acceptability and physico-chemical quality (Ijah *et al.*, 2014; Nwosu *et al.*, 2014; Julianti *et al.*, 2015). The use of composite flour has the effect of reducing wheat flour imports of a country as some sub-Saharan

Africa imports as much as 90% of their wheat flour; the foreign reserves of these countries are therefore increased (Olayimika *et al.*, 2015).

2.3 ORANGE-FLESHED SWEETPOTATOES (OFSP)

OFSP is a biofortified variety of sweetpotatoes and is known to be high in β -carotene (Kidane *et al.*, 2013). It is the first biofortified provitamin A food staple to be developed and promoted for consumption in SSA where most of the landraces, sweetpotato varieties, are white-fleshed or yellow-fleshed and have low β -carotene content (Bouis *et al.*, 2013). This variety was first developed through conventional breeding in the USA and first imported into SSA in 1995 (van Jaarsveld *et al.*, 2006). Since the inception of intense research on OFSP in 1995 as a strategy to combat vitamin A deficiency (VAD), there has been growing utilization of OFSP among the population as well as growing production levels (Jenkins *et al.*, 2015; Andrade *et al.*, 2016). The use of OFSP as a VAD eradication strategy in countries like Guatemala was reported as early as in the mid 1990s (Talsma *et al.*, 2017). It is considered as a staple food crop with the potential to tackle the problems of malnutrition due to inadequate calories and vitamin A deficiency (Tadesse *et al.*, 2015).

Over forty cultivars of OFSP tubers have been introduced in Africa. Some of the sweet potato varieties that have been tried and grown in Kenya include KENSPOT-3, KENSPOT-4, KENSPOT-5, Kabode (NASPOT 10-O), Vitaa, Simama, Pumpkin, Japanese, Kakamega 4, Local check, NASPOT 9-0, Vindolotamu and Vitamu A (Tadesse *et al.*, 2015; Andrade *et al.*, 2016; Talsma *et al.*, 2017). The varieties that have been exploited in puree processing by the sole processor in Homabay County of Kenya, are Vitaa and Kabode (Bocher *et al.*, 2017). The β -

carotene content of OFSP varieties vary as reported by Alam *et al.* (2016) who noted a variation depending on the intensity of the orange colour on the flesh, with lighter orange flesh coloured varieties having lower β -carotene levels.

2.3.1 Nutritional Composition of OFSP Roots

OFSP is rich both in micronutrients and macronutrients (**Table 2.1**). OFSP roots are rich in provitamin A carotenoids than other sweetpotato varieties including cream and white fleshed varieties. β -carotene in its form of *13-cis*, *all trans* and *9-cis* comprise 10-93% of total carotenoid in OFSP roots (van Jaarsveld *et al.*, 2006). Studies by Rodrigues *et al.*, (2016) found OFSP tubers to be high in protein ; its protein is of high biological value. Another study by Dako (Dako *et al.*, (2016) found that OFSP is rich in iron, zinc and calcium in addition to other nutrients such as carbohydrate. A high retention of β -carotene was reported in various OFSP tuber varieties after cooking (Mitra, 2012), this has made OFSP a suitable fortificant for various processed foods. The leaves of OFSP vines are also known to be rich in fiber and protein and other micronutrients such as vitamin A, niacin, riboflavin and vitamin K and are known to be exploited as food in various communities globally (Timothy *et al.*, 2017). Exploitation of OFSP as food thus presents an opportunity of alleviating “hidden hunger” and malnutrition in most parts of the world.

Table 2.1: Nutritional Composition of sweetpotato roots (per 100g portion dry weight)

Nutrient	Orange-Fleshed	White-Fleshed	Cream-fleshed
Protein (g)	1.44-2.50	1.47	2.45
Fat (g)	0.03-0.95	0.16	0.14
Carbohydrates (g)	23.91-33.45	33.27	41.46
Sugar (mg)	102.04-145.60	137	132
Ash (g)	0.87-0.97	0.97	0.97
Magnesium (mg)	12.39-12.36	12.84	14.09
Calcium (mg)	24.40-29.20	27.46	23.55
Iron (mg)	0.54-0.67	0.65	0.65
Total carotenoid (μg)	177.45-994.02	49.32	124.83

Adapted from Lyimo et al. (2010)

2.3.2 Utilization of OFSP

The consumption of OFSP is still low as most of the consumers remain unaware of its nutritive value (Mitra, 2012). OFSP was developed to mitigate against vitamin A deficiency (VAD). Originally, the farming population in SSA was noted to prefer the white or cream-fleshed sweetpotato varieties to the yellow and orange-fleshed varieties largely due to an established market for the white and cream-fleshed varieties (Andrade *et al.*, 2016; Bocher *et al.*, 2016). In all the East African countries, there are programmes that are currently running aimed at promoting the production and utilization of OFSP tubers (AFSA, 2014); the goal of the various programmes is to promote intake of vitamin A especially among the resource-poor population.

OFSP tubers have been utilized as food in their fresh form after cooking, as flour and in the grated and mashed (commonly known as puree) forms (Truong and Avula, 2010; Stathers *et al.*, 2013; Abidin *et al.*, 2015). OFSP flour has a lower bulk than OFSP puree, presenting a convenience for manufacturers to use in terms of transporting it but presents a disadvantage in terms of economic returns and with longer storage (more than two months), β -carotene deterioration is very significant (Stathers *et al.*, 2015). OFSP flour has been used locally at domestic level and in industrial production of bakery products. Some of the bakery products in which OFSP flour is incorporated as an ingredient are cakes, bread, muffins and buns (Sindi *et al.*, 2013). Mashed OFSP has also been used for flour substitution in Golden bread (Jenkins *et al.*, 2015). OFSP is being promoted as a nutrition intervention to tackle VAD and food insecurity in many countries (Alam *et al.*, 2016; Rodrigues *et al.*, 2016; Bocher *et al.*, 2017). In industrial production, OFSP has been used to produce products such as chips, crisps, flour, puree, juice, bread and other bakery products (Dooshima, 2014; Andrade *et al.*, 2016). However, some of these processing techniques such as boiling with lid off reduces bioavailability of β -carotene (Burri, 2011; Jenkins *et al.*, 2015), and are therefore not highly encouraged. In Asian countries, sweetpotato pickles and cubes are in commercial production and are known for their β -carotene rich property (Padmaja *et al.*, 2012). Some of the OFSP roots such as Beta 1 and Beta 2, main varieties grown in Indonesia, are high in moisture thus are not consumed directly as roots but in derivative products (Ginting and Yulifianti, 2015). Such products are processed and serve as functional ingredients. Production and use of OFSP puree as functional ingredients in food processing has been done for over three decades in the United States (US) (Truong and Avula, 2010). OFSP roots have also been exploited as stockfeed as root meal especially in the raising of pigs and in other food processing such as starch extraction in Asian countries (Chang, 2014). The

use of OFSP roots for starch extraction is limited as the dry matter content of these roots are quite low.

2.4 INDUSTRIAL PRODUCTION OF BREAD

The commercial production of bread is carried out in a number of different scales, ranging from artisan bakeries that mainly serve the local community, to large commercial bakeries that supply bread to the entire nation, including in-store and supermarket bakeries (Trinh *et al.*, 2016). The main ingredients of bread are flour, water, yeast, fat and salt, but these vary in ratio resulting into product variation. Other additional ingredients may be added depending on the consumer preferences and the manufacturer's discretion based on market trends (Ishida and Steel, 2014).

The composition of industrially produced bread is about 70–80% gas by volume, the gas cells in the product result from fermentation by yeast, *Saccharomyces cerevisiae* (Trinh *et al.*, 2016). The gas cells are responsible for the quality parameters of bread such as texture and brightness of the crumbs, absorbance and loaf volume. Softer breads are perceived as fresher than firm ones which are seen as stale (Fadda *et al.*, 2014). Various factors determine the firmness of the crumb, including the density and quantity of the crumb and the volume and distribution of gas cells in the crumb (Onyango, 2016). Most of the industrial production of bread uses wheat which happens to be composed of carbohydrates, proteins, minerals, fats and water (Kumar, Khatkar and Kaushik, 2013). Industrial bread making equipment typically processes batches of at least 200 kg of raw ingredients (Trinh, Campbell and Martin, 2016).

Bread production is divided into five major stages as diagrammatically illustrated in **Figure 2.1**:

1. Dough Formation: The raw ingredients undergo mixing, binding and shaping the dough pieces.
2. Proofing: The dough is subjected to appropriate temperature and humidity for yeast fermentation to occur thus making the dough to rise.
3. Baking: The dough is heated in an oven at high temperature for most moisture in the dough to evaporate and baking into bread.
4. Cooling: The temperature of the bread is lowered to ambient temperature.
5. Slicing, packaging and distribution: The bread is made ready to be delivered to the consumers and also this serves to enhance the shelf-life and prevent contamination.

Composite flour would result in reduced content of wheat flour with a corresponding decline in the gluten content of the flour. Decline in the gluten impacts on the physical attributes of the bread especially the loaf volume of the bread. Nwanekezi (2013) reports that wheat flour content equal to or less than 8% makes it virtually impossible to produce a quality bread.

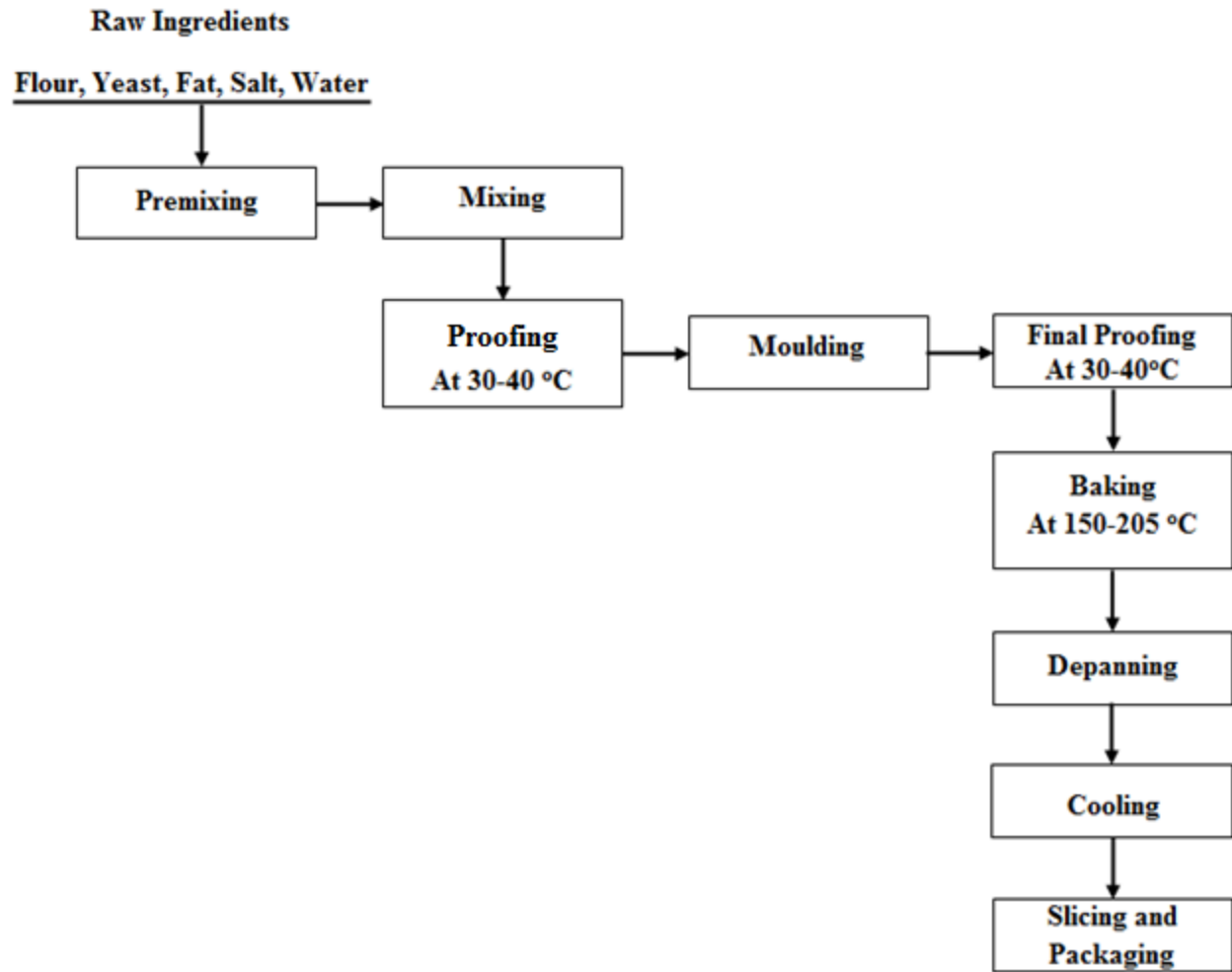


Figure 2.1: Schematic representation of bread baking process

2.4.1 Consumption of Bread

Wheat bread is consumed globally but with varied recipe (Othman Alj, 2017). In Europe, wheat flour bread is the single most consumed wheat flour product and has inevitable presence in the daily diet of most European families (Nwanekezi, 2013). In most of the SSA countries, bread is an alien product but whose production has been done over ages that the recipe has been customized. The increase in bread consumption globally can be attributed to increasing global

population, rapid urbanization in developing countries and changing eating habits (Adeniji, 2013).

With the introduction of other breakfast cereals in the Western World, a decline in bread consumption has been realized whereas in SSA an increase has been noted (Bockstaele *et al.*, 2009). Even with the declining trends, Turkey and Bulgaria have maintained stable consumption patterns and still rank highest in per capita bread consumption in Europe; 104kg and 95kg per capita, respectively (Eglite and Kunkulberga, 2017). According to World Bank Statistics in 2010, of all the SSA countries, South Africa has the highest proportion (11%) of its national household population in the higher consumption segment of bread (above \$23.03 per capita per day) (World Bank, 2018). Mason *et al.* Mason *et al.*, (2012) reported an increasing affordability of bread than both rice and maize in Kenya; a corresponding increase in the consumption of bread has also been noted. The ever increasing presence of bread in the diet of especially SSA population cannot be ignored or rather presents an opportunity of ameliorating the nutritional status of this population.

2.4.2 Shelf-Stability of Bread

Stability of bread in storage is defined by moisture content, microbial growths and staling (Kwaśniewska *et al.*, 2014) . The microbiological and physico-chemical changes that occur in bread in storage affect the shelf-stability of the bread. The physico-chemical changes in bread result into firming of bread in a process known as bread staling (Melini, 2018). Bread can either be a low acid or a high acid food depending on the ingredients and processing technique: this is a determinant of stability of such bread in storage (Smith *et al.*, 2004). Breads produced under various processing techniques have varied shelf stability; Bhise and Kaur (2014) reported a shelf

life of 5 days whereas Latif *et al.* (2005) reports a lesser days, 3 days; all this depends on the manufacturing techniques and the additives.

The shelf-life of bread is classified into physical, chemical and microbiological shelf-life (Smith *et al.*, 2004). The shelf-life of bread depends on processing, packaging, formulation and storage conditions (Galic *et al.*, 2009). Preservation of bread seeks to control rancidity, moisture migration, development of off-flavours, crystallization, grittiness and structural weakness in bread (Bhise and Kaur, 2014). Bread preservation has been done using various preservation techniques including physical methods such as infrared, ultraviolet light, ultra high pressure (UHP) and microwave heating; chemical preservatives such as sodium acetate, acetic acid, potassium acetate among others; and bio-preservation as in the case of sour bread (Melini, 2018). Advances in research are exploiting the use of nanotechnology and packaging to improve shelf-life of bread (Melini, 2018).

2.5 PRODUCTION OF ORANGE-FLESHED SWEETPOTATO PUREE BASED BREAD

OFSP flours and puree have been documented to have been used to produce OFSP based bread (Omodamiro *et al.*, 2013; Low *et al.*, 2017). As early as in the 1990s, OFSP had been incorporated into bread production in Peru in grated form as little sun was available for sundrying to produce flour (Sheikha and Ray, 2015). Incorporation of OFSP in wheat products also has economic advantages as it was the case of cassava that helped reduce reliance on imported expensive wheat flour. The incorporation of either OFSP flour or puree in bread aims at improving the β -carotene content of bread (Kamal *et al.*, 2013; Bonsi *et al.*, 2014). The use of puree in the substitution of flour in bread baking presents an economic and nutritional advantage

as the flour has a lower conversion rate of 4.5-5kg to produce a kilogram of flour compared to 1.3-1.6 kg of fresh root required to produce a kilogram of puree and with storage beyond two months, significant losses of β -carotene is noted in OFSP flour (Low and Jaarsveld, 2008; Bocher *et al.*, 2017; Low *et al.*, 2017). This is because OFSP roots are high in moisture content and the dry matter may be as low as 30%, thus alternative economical ways such as puree or paste for bakery products are recommended (Mwanga and Ssemakula, 2011; Ginting and Yulifianti, 2015; Alam *et al.*, 2016). A Study done by Muzhingiri (2016) to assess the effect of baking on the β -carotene content of OFSP flour bread and OFSP puree bread found that OFSP puree based bread had a high β -carotene content of 3.14 mg/100g dry weight (DW) while that from OFSP flour had 0.08mg/100g DW; two slices of bread from OFSP puree could actually meet almost 30% of the RDA for vitamin A in children aged 1–3 years old. This clearly shows the nutritional advantages posed by use of puree over the use of flour.

The production of OFSP puree by the sole processor in Kenya follows the diagrammatic representation shown in **Figure 2.2**. The puree is usually stored frozen for later use thus preserving most of its chemical and physical properties.

OFSP roots are highly perishable (starts to rot 1-2 weeks after harvest), thus the conversion to puree enhances the shelf stability and product utilization (Andrade *et al.*, 2016). Originally, the puree was subjected to freezing for its preservation. However, through latest research advances in puree preservation, the shelf-stability of the puree in storage at ambient temperatures has been enhanced. The puree is preserved using potassium sorbate, sodium benzoate and citric acid and undergoes vacuum packaging enabling it to have a shelf-life of 4 months at 23°C (Bocher *et al.*, 2017). Potassium sorbate, sodium benzoate and citric acid each singly has antimicrobial effect

(Su *et al.*, 2014; Biswas *et al.*, 2015). Synergism has been shown to increase the effectiveness of food preservatives (Baljeet *et al.*, 2015). The use of potassium sorbate, sodium benzoate and citric acid as preservatives has been proven to have no effect on the β -carotene retention both in puree and bread produced (Muzhingi, 2016). Vacuum packaging has been proven effective as studies of vacuum packaging of sweetpotato puree in America date back to early millennia (Steed *et al.*, 2008).

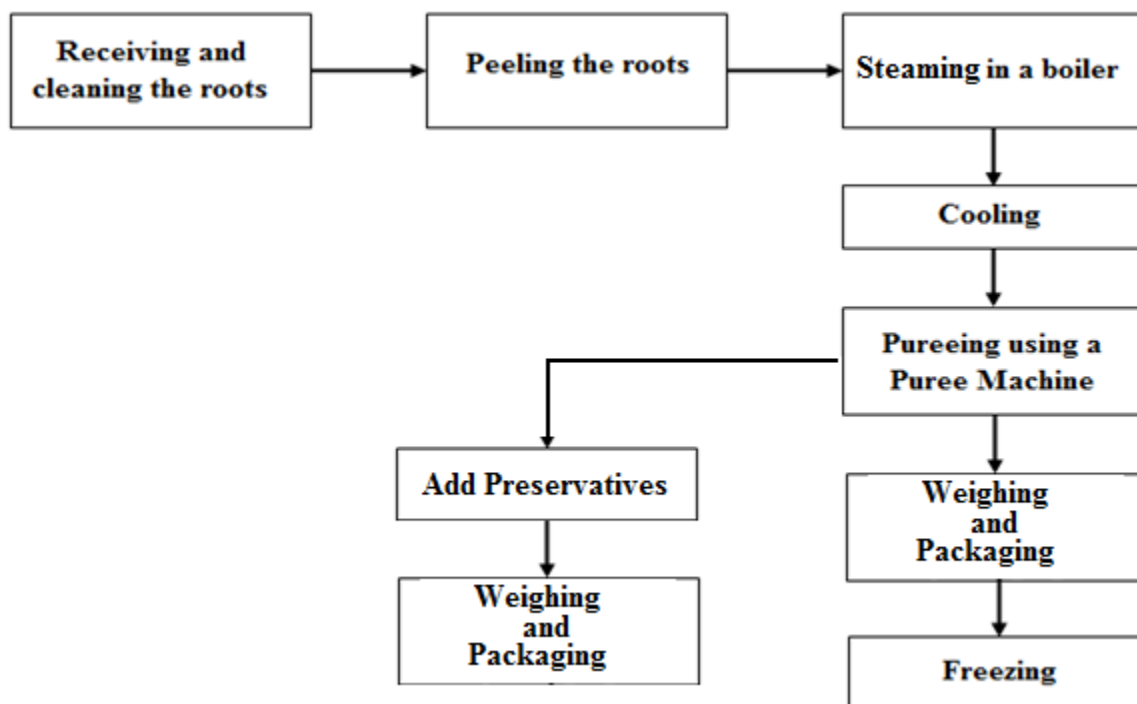


Figure 2.2: Schematic representation of Puree Processing

In developed countries such as European countries and the US, nutrition awareness has served to spur demand for OFSP thus increasing production of novel foods (Gruneberg *et al.*, 2012). Utilization of OFSP as a fortificant in various foods in these countries has been a practice over time. OFSP puree has been used as an ingredient in commercial bread production in Kenya as a substitute for wheat flour with 40% being the optimal substitution (Bocher *et al.*, 2016; Bukania,

2016). In Ghana, the bread is on the roll out for industrial production under the trade name “vitabread” (Abidin *et al.*, 2015). In Rwanda the substitution has been done in the proportions ranging from 20 to 45% (Bukania, 2016). Studies have documented that 30% OFSP puree substitution is the minimum level that possibly provide significant β -carotene levels (Sindi *et al.*, 2013; Muzhingi, 2016) justifying the fortification of bread using OFSP puree. The use of OFSP puree as a substitute for flour has provided both economic and nutritional advantages (Abidin *et al.*, 2015). The incorporation of OFSP puree in bread targeted the high end market who could not easily access the OFSP roots and benefit from its β -carotene rich property (Sindi *et al.*, 2013). The optimal product formulation is as shown in **Table 2.2**.

The use of OFSP puree subjected to cold-chain storage presents a challenge in terms of additional costs as the storage demands electric power and additional equipment if the long term production is to be sustained. Researchers have developed a shelf-storable puree treated with preservatives (sodium benzoate, potassium sorbate and citric acid) which has a shelf-stability of 4 months (Bocher *et al.*, 2017). The use of preservatives especially on OFSP puree should be with care as studies have shown that some preservatives can affect the sensory quality of the product (Shahnawaz *et al.*, 2012). The use of these preservatives has been found to transfer antimicrobial properties such as antifungal properties by potassium sorbate and others to some extent improve on the sensory quality of products if applied appropriately (Malhotra *et al.*, 2015).

Table 2.2: Optimal formulation for OFSP bread

Ingredients	Proportions
Wheat flour	60%
Puree	40%
Sugar (based on puree and flour weight)	1%
Salt (based on puree and flour weight)	1.5 %
Yeast (based on puree and flour weight)	1.2%
Vital gluten (based on puree weight)	2%
Dough Improvers (based on puree and flour weight)	0.5%
Shortening fat (based on puree and flour weight)	0.4%

Adapted from Bukania (2016)

Bread made from shelf-storable puree is yet to be evaluated in terms of its physico-chemical and sensory attributes and shelf-stability. The adoption for use of this puree in bread production has advantages including high economic returns as there would be reduced spoilage of roots during bloat, consistency of supply of puree to bakeries and reduced bread production costs as the shelf storable OFSP puree has a shelf-life of up to four months at ambient storage (23°C) (Bocher *et al.*, 2017). The use of shelf-storable puree is also a better alternative for bakers who lack puree processing plants or cannot access fresh puree.

2.5.1 Sensory and Nutritional Quality of OFSP Puree Bread

Promotion of OFSP roots as a functional food in India proved a successful strategy towards eliminating VAD while cost-effectiveness is met (Attaluri and Ilangantileke, 2007). Extending such efforts to other resource poor countries has the potential of improving nutrition status of various communities. Bread made by substituting 38% of the wheat with OFSP puree was found to be high in vitamin A (135 RAE/110 g) and had a deep-yellow colour which was highly acceptable among consumers in Mozambique (Andrade *et al.*, 2016). Other studies done in Rwanda showed that there is great consumer preference of bread made of composite mixture of 30% OFSP-puree and 70% wheat-flour to that made of 100% wheat-flour (Sindi *et al.*, 2012). A study by Bonsi *et al.* (2016) found that 30% wheat flour substitution had the highest overall acceptability but texture had the lowest acceptability, increasing flour substitution increased firming in bread.

Incorporation of OFSP puree into bread baking can serve to enrich the bread energy and nutrients such as vitamins (pro-vitamin A) and minerals (Ca, P, Fe, Zn and K) and also add natural sweetness, color, flavor and dietary fiber (Mitra, 2012; Laelago *et al.*, 2015). The “vitabread” has a trans β -carotene content of 1.333 mg/100 g meeting about 21% of daily requirement (1,300 μ g RAE/day) of nursing mothers (Awuni *et al.*, 2017). Studies in the US have proven OFSP roots as worthy fortificants to help ameliorate nutrition status (Burri, 2011): in SSA bread offers that opportunity. OFSP puree also gives the bread a golden yellow colour thus resulting in high consumer acceptability (Wheatley and Loechl, 2008). This has encouraged its wider use as compared to OFSP flour in production of bread and other bakery products. Various studies in India, Nicaragua, Mexico, Philippines and India among many others have shown a general likability of products made from OFSP in novel recipes including bread (Talsma

et al., 2017). Enhancing production and introduction of more novel products in which OFSP has been incorporated provides an opportunity for its increased utilization as a functional food.

2.5.2 Spoilage and Safety of Orange-Fleshed Sweetpotato Puree Bread

Baking ensures that the bacteria and mould spores which were in the flour are destroyed. However, some of the bacteria spores are known to survive the heat treatment and under favourable conditions germinate to cause roping of the bread (Saranraj and Geetha, 2012). *Bacillus* sp. are known to form spores which can withstand the baking process (Rumeus and Turtoi, 2013; Ijah *et al.*, 2014). OFSP puree based bread is subject to microbial contamination from handling of the puree and the wheat flour. The use of contaminated ingredients such as flour or OFSP puree would easily cause microbial contamination of the bread (Madani *et al.*, 2016). It is, therefore, important to ensure that the raw materials are free from mycotoxin or fungal contamination to minimize occurrence of fungal contamination in bread. Post-thermal processing contamination is also possible (Chavan and Chavan, 2011). Proper hygiene in food handling of food processing equipment ensures limitation of microbial contamination of the product (Al-Bahry *et al.*, 2014).

OFSP puree based bread also undergoes spoilage by staling which is the commonest spoilage in bread (Hiroaki *et al.*, 2014). Bread staling involves modifications of the product matrix, both the macroscopic and the molecular structures. In the macroscopic structure, staling affects the firmness of the crumb, deterioration of the product flavour and softening of the crust while in the molecular structure, it causes retrogradation and crystallinity of starch, water redistribution within the molecules of bread, decreases the amount of soluble amylose and changes the gluten (wheat protein) network and the interactions between gluten protein and starch granules in wheat

bread (Kwaśniewska *et al.*, 2014). The staling of bread is as a result of the slow change of the starch at temperatures below 55°C from a moistened amorphous form to a hard less moistened crystalline form. This change causes the rapid hardening and shrinking of starch granules away from the gluten skeleton resulting into crumbliness (López *et al.*, 2013). It is important to ensure proper preservation to avoid bread staling as it results into deterioration of quality (Lee, 2012).

2.5.3 Marketability of OFSP-puree Bread

Commercialization of a product should be demand driven rather than a push by a “champion”. Other factors can come into play in commercialization of a product such as consumers’ growing awareness and demand for healthier products (Sindi *et al.*, 2013). The OFSP puree based bread is commercially available in Kenya through selected retail chains (Bocher *et al.*, 2017). OFSP puree bread has been in commercial production in Japan since the 1990s where it happens to be a renowned functional food consumed by a number of people (Truong and Avula, 2010). A study done in Kenya to evaluate marketability of OFSP based products, showed that consumers were willing to pay for such products (Low *et al.*, 2017), thus a proof of likelihood of success in adopting shelf-storable OFSP puree based bread.

A study done among consumers in Kenya revealed that the willingness to pay for OFSP puree based bread by the consumers is high owing to its nutritional value (Bukania and Muzhingi, 2017). OFSP bread developed from shelf storable puree, with evaluation of its nutritional content, can have the market demand created through awareness creation (Stathers *et al.*, 2015). With the success of marketability of shelf-storable OFSP puree based bread, the economic gains can be realized across the OFSP supply and value chains. This would be a great incentive for farmers to continue with the production of OFSP roots.

2.6 CONCLUSION

The use of OFSP puree in bread baking has been an avenue for food fortification and product diversification. OFSP puree based bread can enhance vitamin A intake among the population especially the consumers of products in the high end markets. The challenge posed by the use of fresh OFSP puree in the production of bread can be overcome by the use of shelf-storable preservative treated OFSP puree. However, the physico-chemical properties, shelf-stability and sensory attributes of the bread produced from the shelf-storable puree require systematic evaluation in order to establish a substantive argument for its use. With the high consumption of bread among populations, OFSP puree based bread can be part of efforts towards improving the vitamin A status.

CHAPTER THREE: PHYSIOCHEMICAL CHARACTERISTICS OF ORANGE FLESHED SWEETPOTATO (*IPOMOEA BATATAS*) SHELF-STORABLE PUREE-WHEAT COMPOSITE BREAD

3.1 ABSTRACT

The study sought to evaluate chemical preservative treated OFSP puree (shelf-storable) as an alternative to fresh puree in bread production and its impact on the physico-chemical attributes of the bread. Two treatments of shelf-storable OFSP puree: treatment 1 and 2 with 0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid and 0.2% potassium sorbate + 0.2% sodium benzoate + 1% citric acid respectively, were incorporated into bread at 30% and 40%. Sampling of the purees was done monthly for a period of four months for incorporation into bread and for biochemical and biophysical testing. The β -carotene content of treatment 1 and treatment 2 shelf-storable OFSP purees decreased with storage time from 12.75 ± 1.66 to 6.93 ± 0.50 mg 100g^{-1} dry weight and from 12.75 ± 1.66 to 5.45 ± 0.25 mg 100g^{-1} dry weight from baseline to month 4 respectively. Shelf-storable puree bread had a vitamin A content ($173 \mu\text{g RAE } 100\text{g}^{-1}$ dry weight) that could meet half the daily requirement of children aged 1-3 years. The results for proximate analysis showed no significant differences ($P>0.05$) in the crude protein, crude fat, carbohydrate and crude fiber contents of the breads. Both treatments of shelf-storable OFSP puree breads had higher crude ash content, 2.07 ± 0.13 to 2.24 ± 0.26 g 100g^{-1} , than control wheat bread ($P<0.05$). Incorporation of OFSP puree into bread increased the moisture content, ranging from 29.63 to 31.92 g 100g^{-1} ($P< 0.05$). Incorporation of shelf-storable OFSP puree into bread had no significant ($P>0.05$) effect on the loaf weight, volume and specific volume. Shelf-storable puree, notwithstanding the level of preservatives used, proved an alternative to fresh puree in

enriching bread with beta-carotene at 40% wheat flour substitution up to a period of three months of its storage.

3.2 INTRODUCTION

Considerable research on food-based strategies using Orange Fleshed Sweetpotato (OFSP) to combat Vitamin A Deficiency (VAD) in sub-Saharan Africa (SSA) have largely been successful (Low *et al.*, 2015). However, one of the challenges of biofortification is the sustainable adoption of the nutritious crop by target populations. Creation of market opportunities for OFSP through value chain development can result in sustainable adoption and consumption of vitamin A rich OFSP (Wachira, 2012). This consumption can result in reductions of VAD and malnutrition among smallholder farmer households, especially young children and women at child bearing age (Jenkins *et al.*, 2015). In Africa, there is a rapid growth in the middle class that is highly educated and are demanding local, safe and nutritious and modern foods, in line with the changing food systems. There is a need to provide convenient, affordable, safe and nutritious foods to the growing urban poor in Africa. Therefore, innovations around OFSP as food products and as versatile ingredients provide opportunities to address malnutrition in Africa.

There are no crops that can match the baking properties of wheat flour, thus substitution and blending of flour is encouraged (Ohimain, 2014). Many African countries import close to 100% of their wheat flour requirements for baking, thus losing the much needed foreign currency (Nwanekezi, 2013). In Nigeria, the government has promoted cassava, an indigenous crop, as an ingredient in bread baking in efforts to reduce dependence on imported wheat (Adeniji, 2013). This was done through a law that required incorporation of 10% of cassava in wheat flour by all millers. Composite breads have been used globally to improve the macronutrient and

micronutrient properties of the breads, but challenges have been reported in physico-chemical attributes. Other indigenous and less expensive crops that have been exploited for partial substitution of wheat flour include legumes, maize, cocoyam, bread fruit and sorghum (Malomo *et al.*, 2011). Therefore, the use of composite flours in bakery is aimed at improving profitability by reducing ingredient and production costs, improving nutritional quality of bread, while limiting the negative effects on the physical and sensory attributes.

Currently, in Kenya, OFSP puree is cheaper than OFSP flour (Bocher *et al.*, 2017). Unlike OFSP flour, the incorporation of OFSP puree into bread has both health and economic benefits. The substitution of wheat flour with OFSP puree increases the β -carotene composition, a provitamin A compound, of the bread as OFSP roots are rich in β -carotene as high as $6.9\text{mg } 100\text{g}^{-1}$ dry weight (Bonsi *et al.*, 2014). OFSP puree incorporation into bread formulation results in reductions in sugar, fat, water and colorants, thus reducing the production costs. Adoption of locally grown and produced OFSP puree will reduce the dependency on imported wheat for bakery products in SSA. Sensory and consumer acceptance studies have also shown that OFSP puree products are marketable and profitable (Sindi *et al.*, 2013).

In the USA, OFSP puree processing is advanced and uses microwave aseptic technology (Steed *et al.*, 2008). This shelf storable OFSP puree has a shelf-life of 12-24 months. This advanced technology is yet to be adopted in SSA. Currently, OFSP puree processors in SSA use a cold chain which is expensive (Bocher *et al.*, 2017). Studies from pumpkin and peach puree shows that chemical preservatives can be used safely to extend the shelf-life of fruit and vegetable puree cost effectively (Gliemmo *et al.*, 2014). Sorbic acid and its potassium salt are used as antimicrobial agents in a wide range of foods, including processed vegetable products (Aneja *et*

al., 2014). Citric acid is generally added to fruit formulations to maintain their pH < 4.6, because high-acid foods require less heat treatment to ensure stability. Citric and ascorbic acids are not only used to reduce fruit pH values but also to control browning in fresh and processed fruit products (Ioannou and Ghoul, 2013). Different combinations of citric acid, sorbate and benzoate together with vacuum packing were shown to increase the shelf-life of OFSP puree by 3-6 months in Kenya and produced safe products for human consumption (unpublished data). This study evaluated the physical and chemical characteristics of composite breads developed from shelf-storable OFSP puree to provide an evidence-based justification for its use as an alternative to the fresh puree, thus enhancing the adoption of the technology with reduced costs.

3.3 MATERIALS AND METHODS

3.3.1 Sample Preparation

Shelf-storable OFSP puree made from Kabode variety of OFSP tubers was obtained from the sole processing plant in Homa Bay County. Wheat flour, Exe (Unga Limited, Kenya) was purchased from a retail outlet. Salt (Kensalt Limited, Kenya), Sugar (South Nyanza *Sugar Company* Limited, Kenya), baking powder (Kapa Oil Refineries, Kenya), baking soda (R H Devani Limited, Kenya) and yeast (Foodplus, Kenya) were purchased from the local shops in Homa Bay County. The OFSP puree was treated in two ways: treatment 1 had 0.5% potassium sorbate + 0.5% sodium benzoate + 1% Citric acid while treatment 2 had 0.2% potassium sorbate + 0.2% sodium benzoate+1% Citric acid. The control fresh puree was stored frozen at -20°C for a period of four months levels that have proved effective for preservation of the puree as reported by Musyoka *et al.* (2018). Treatment 1 and 2 purees were stored at ambient temperatures (15-23°C) away from light. All the stored samples were packaged in sealed transparent polyethylene

plastic bags (300 gauge). The purees were sampled monthly for use in bread baking for a period of four months.

Both treatments were used to make bread with wheat flour substitution of 30%, which was the minimum level known to provide adequate quantity of beta-carotene (Sindi *et al.*, 2013; Muzhingi *et al.*, 2016), and 40% which is the practice in the Kenyan market. Sodium bicarbonate (baking soda) was used to neutralize acidity of shelf-storable puree according to Groves and Brill (2015) where baking soda was determined based on the following formula:

$$\text{Soda (g)} = \frac{\text{Acid (g)} \times \text{Neutralization value}}{100}$$

The neutralization value that was used was 159 as stated by manufacturer (Univar Food Ingredients, United Kingdom).

The bread samples were made as shown in **Figure 3.1**. The ingredient mixes were as shown in **Table 3.1** which was adopted from recipes by Antonio Magnaghi of Euro-Ingredients Limited, Nairobi, Kenya. Water was first added to flour, puree, sugar, bread improver and baking powder mix. Fat, salt and baking powder were then added and kneading done. The dough was subdivided into 460g sizes and transferred into an already greased aluminum loaf pan. Proofing was done for 45 min at 40°C and 85 % RH. Baking was done for 30 minutes at 200°C then removed from the pan, cooled overnight, and packaged in a polythene bag and stored at 23°C. The samples were transported in frozen form for physico-chemical analysis in the Food and Nutritional Evaluation Laboratory (FANEL), Biosciences for East Central Africa (BecA) Hub at the International Livestock Research Institute (ILRI), Nairobi, Kenya.

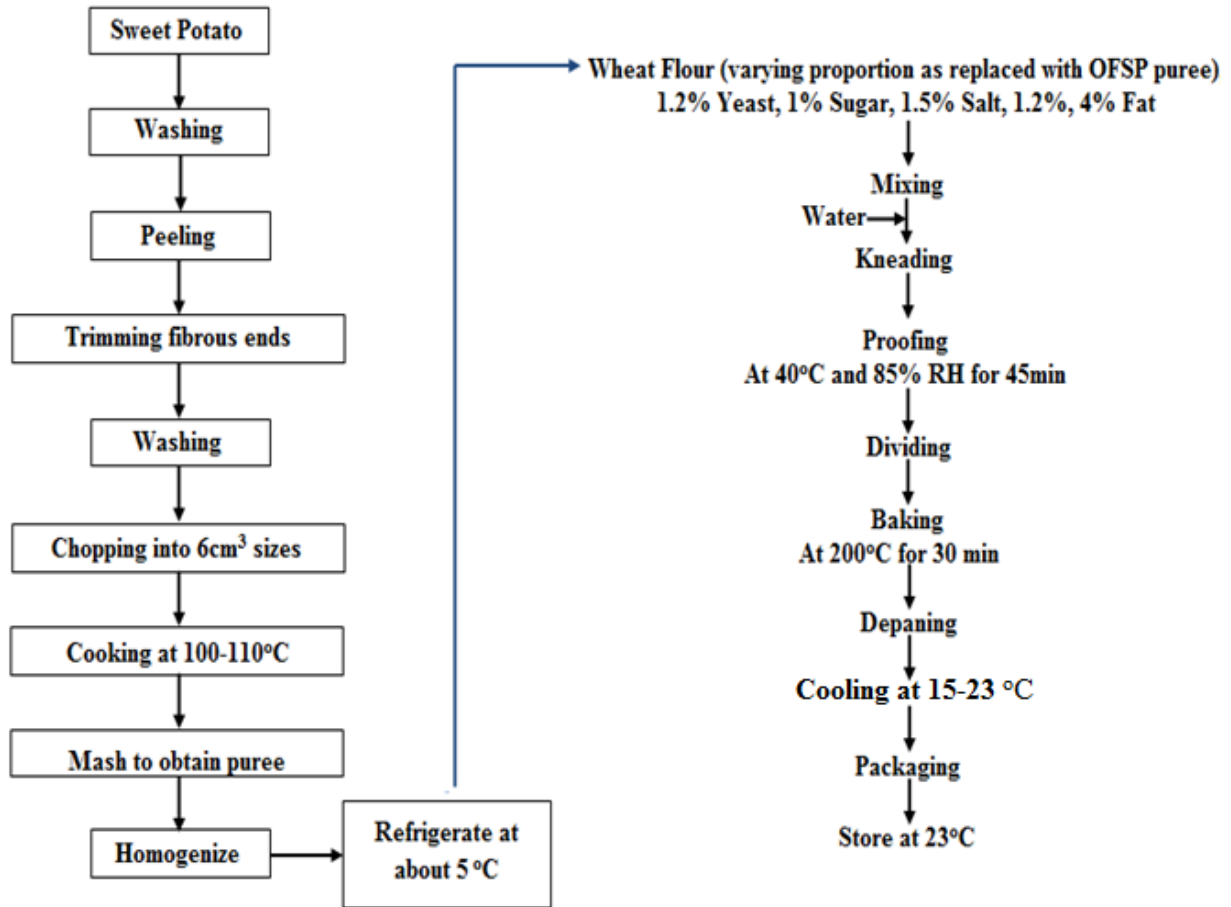


Figure 3.1: Schematic Representation of Baking of Bread Samples

Table 3.1: Ingredients for shelf-storable OFSP puree-wheat composite bread

Ingredient (g)	Wheat bread	30% Fresh puree bread	40% Fresh puree bread	30% T1 SS bread	40% T1 SS bread	30% T2 SS bread	40% T2 SS bread
Flour	2500	1750	1500	1750	1500	1750	1500
Puree	0	750	1000	750	1000	750	1000
Sugar **	125	75	75	75	75	75	75
Salt**	25	25	25	25	25	25	25
Fat**	100	75	75	75	75	75	75 (3)
Yeast**	37.5	50	50	50	50	50	50
Bread Improver**	0	7.5	7.5	7.5	7.5	7.5	7.5
Baking powder**	0	0	0	12.5	12.5	12.5	12.5
Baking soda	0	0	0	11.9	15.9	11.9	15.9

*SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid in puree. **Ingredients added as proportion of the total quantity of wheat flour + OFSP puree composite.

3.4 ANALYTICAL METHODS

3.4.1 Determination of Proximate Composition of Puree and Bread

Proximate composition of bread and puree were determined as per AOAC (2012) methods in terms of moisture content (method 925.10), crude protein (method 920.87), crude fiber (method 920.86), crude fat (method 922.06C), crude ash (method 923.03) and carbohydrate content (by difference method).

3.4.2 Determination of Beta-Carotene Content in OFSP Puree and Bread

The β -carotene content was determined using high performance liquid chromatography (HPLC) by modification of the method described by Kurilich and Juvik (1999). The crumb and crust of each sample were separately subjected to analysis. Approximately one gram of the sample was accurately weighed into a 25ml tube. For the OFSP puree, one gram of homogenized puree samples was weighed in 25ml tube. To it, 5ml of methanol was added and vortexed for a minute to mix. The sample was incubated at 70°C for 10 minutes in a water bath followed by centrifugation at 800x g for 10 minutes. The liquid part was decanted slowly into a 25ml volumetric flask. To the remaining residue, 5ml of tetrahydrofuran was added, vortexed for a minute and centrifuged at 800x g for 10 minutes. The liquid part was decanted into the 25ml volumetric flask. Extraction with tetrahydrofuran (5ml each time) was repeated thrice. The 25ml volumetric flask was filled up to the mark with tetrahydrofuran and mixed by shaking. Upon mixing, 2ml of the extract was transferred to a clean 25ml tube; 4ml HPLC hexane and 3ml deionized water were added. The mixture was vortexed for a minute and centrifuged at 800x g for 10 minutes. The upper phase was transferred into a 15ml tube using a Pasteur pipette. The solvent was evaporated in an N-evaporator with temperature maintained below 40°C. The dry

residue was reconstituted with methanol: tetrahydrofuran mixture (85:15). An ml of the reconstituted mixture was transferred into HPLC vials. The HPLC systems consisted of a Shimadzu CBM -20A Prominence Bus Module, SPD -M20A Prominence Photo Diode Array (PDA), DGU 20A5R Prominence Degasser Module, SIL 30AC Nexera Autosampler, two Nexera X2 LC 30AD pumps, a YMC Carotenoid S-3 μ m, 150 x 3.0 mm I.D column, and Shimadzu Lab Solutions data management software. The HPLC solvent gradient elution and time program was as previously published (Yeum, Booth et al. 1996). The HPLC mobile phase was methanol: methyl-tert-butyl ether: water (83:15:2, v/v/v, with 1.5% ammonium acetate in the water, solvent A) and methanol: methyl-tert-butyl ether: water (8:90:2, v/v/v, with 1% ammonium acetate in the water, solvent B). The gradient procedure at a flow rate of 1 ml/minute was as follows: 1) 90% solvent A and 10% solvent B for 5 minutes; 2) a 12-minute linear gradient to 55% solvent A; 3) a 12-minute linear gradient to 95% solvent B; 4) a 5-minute hold at 95% solvent B; and 5) a 2-minute gradient back to 90% solvent A and 10% solvent B. Carotenoids were monitored at UV maximum absorption of 450 nm and DAD spectral data from 250 to 550 nm were stored to examine spectrum peaks for carotenoids. Carotenoids were quantified by determining peak areas in the HPLC chromatograms (**illustrated in Figure 3.2**) calibrated against known amounts of standards. Concentrations were corrected for extraction and extraction and handling losses by monitoring the recovery of the internal standard (Echinenone).

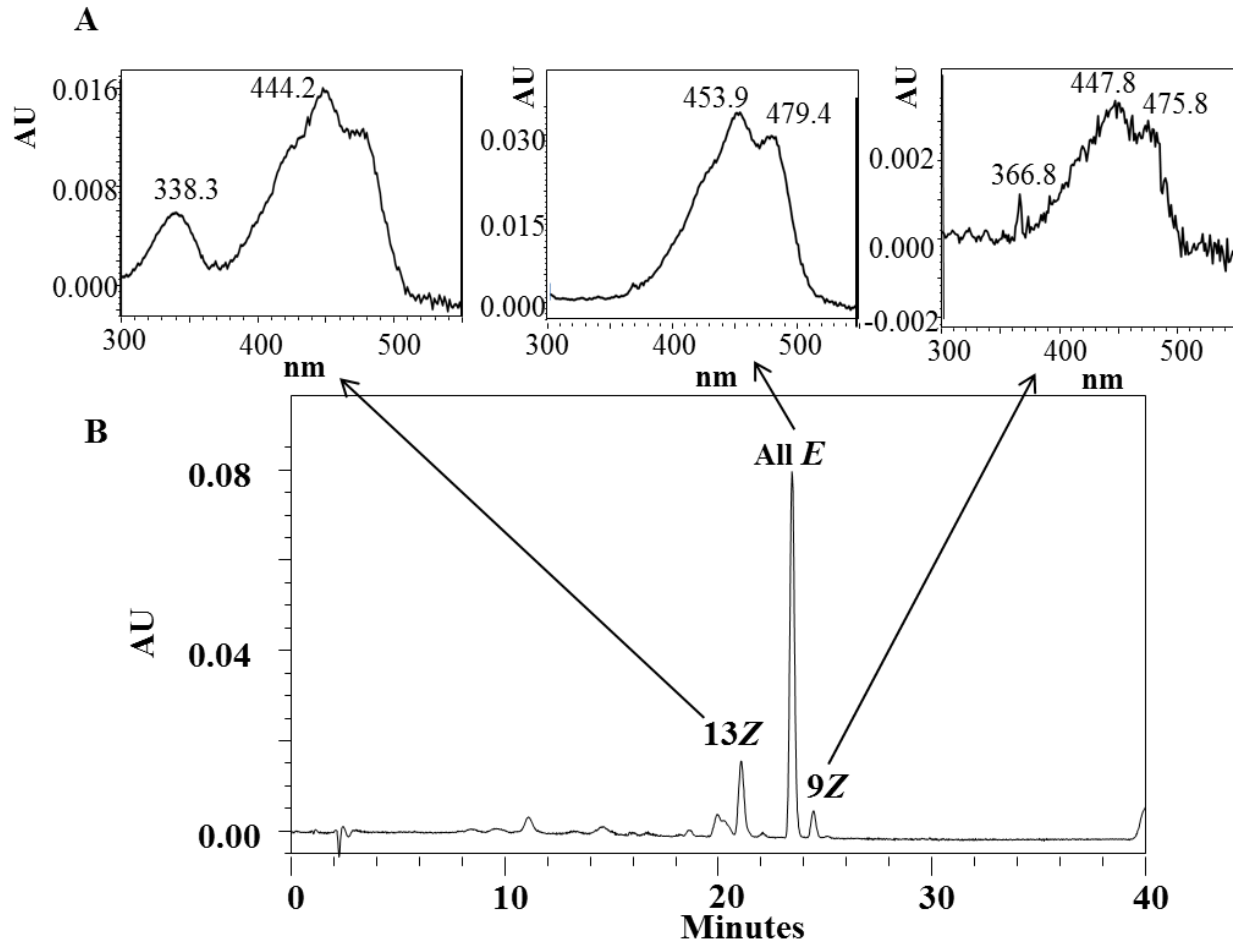


Figure 3.2: HPLC spectra (A) and chromatogram (B) for isomers of β -carotene obtained for samples of shelf-storable OFSP puree bread

3.4.3 Determination of Bread Volume

Loaf volume was determined as per the seeds displacement method described by Nwosu *et al.* (2014). Simsim seeds were placed into a container to determine their volume. The seeds of known volume were poured out and bread put into the container then refilled with simsim seeds. The volume of the extra simsim seeds was determined to represent the volume of bread.

3.4.4 Determination of Loaf Weight

The loaf weight was determined according to the method described by Nwosu *et al.* (2014) by weighing the loaf in calibrated weighing machine.

3.4.5 Determination of Specific Bread Volume

The specific volume of the loaf was determined according to the method described by Nwosu *et al.* (2014) and calculated as follows:

$$\text{Specific volume (cm}^3\text{/g)} = \frac{\text{Loaf Volume}}{\text{Loaf Weight}}$$

3.4.6 Statistical Analysis

The data was analyzed in Genstat version 15. Descriptive statistics such as mean and standard deviation of proximate characteristics, β -carotene content and physical attributes were obtained. The proximate composition and β -carotene content of OFSP puree was analyzed using one way ANOVA while β -carotene content of bread results were analyzed using two-way ANOVA in test for statistical significance. LSD was used to separate means that were significantly different at $p < 0.05$.

3.5 RESULTS AND DISCUSSION

3.5.1 Nutritional Composition of OFSP Puree

The puree used in the current study had high moisture content $68.59 \pm 0.17\text{g } 100\text{g}^{-1}$ while other proximate components were low (**Figure 3.3**). Another study by Selvakumaran *et al.* (2017) that evaluated the moisture content in OFSP puree found a higher value, $75.0\text{g } 100\text{g}^{-1}$. The difference observed can be explained by the variation of moisture content among varieties and as affected by various cooking techniques. OFSP roots which are processed into puree are known to be high

in moisture content, as high as 72.96 g 100g⁻¹ has been reported by Alam *et al.* (2016). The crude ash content of the OFSP puree was 1.36 ± 0.06 g 100g⁻¹ which was comparable to 1.39 ± 0.18 g 100g⁻¹ obtained in a study by Haile and Getahun (2018). The crude protein content of the puree was found to be 1.39 ± 0.17 g 100g⁻¹. This value agrees with values reported by Low *et al.* (2015) who concluded that OFSP purees are low in protein. Meta-analysis of different studies that focus on proximate analysis of orange-fleshed sweetpotato puree may show a variation and this is explained by the different genotypes and processing techniques used (Kelechukwu *et al.*, 2016).

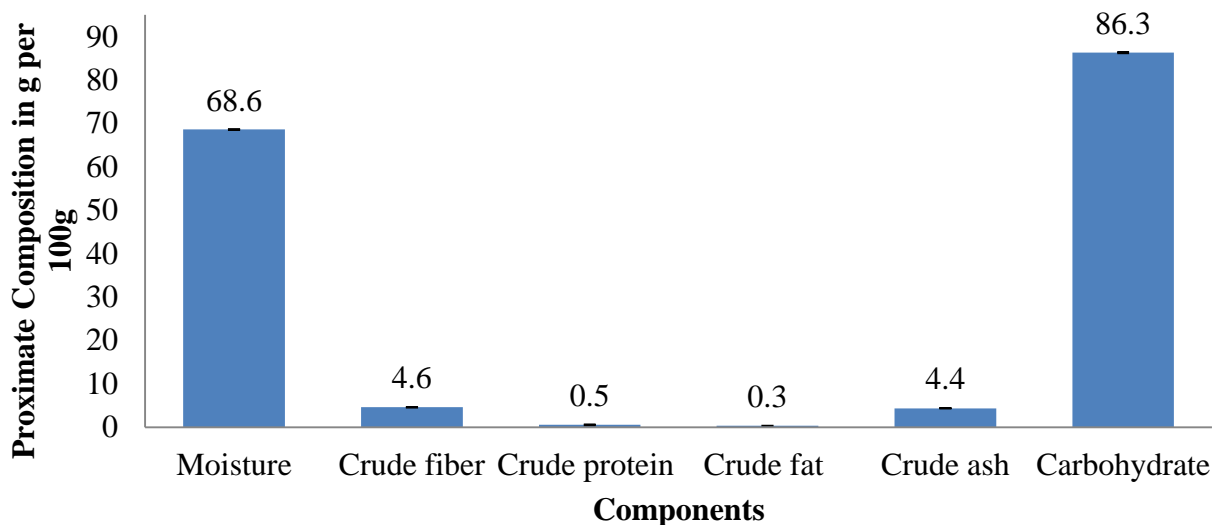


Figure 3.3: Proximate composition of OFSP puree. The bars indicate the standard error of means.

β-carotene content of the OFSP puree was significantly ($p < 0.05$) reduced by the fourth month in both treatments of shelf-storable OFSP puree, 45.6% and 57.3% reduction for treatment 1 and treatment 2 shelf-storable OFSP purees respectively (**Table 3.2**). However, this would possibly still be exploited in fortification as levels of 100-1600 μg RAE have been used in interventions

aimed at eradicating VAD (Low *et al.*, 2007; Nzamwita, Duodu and Minnaar, 2017). The purees were high in All-trans β -carotene, ranging from 11.27 to 4.59 mg 100g⁻¹, which is a known isomer of β -carotene. In storage, β -carotene loss can occur through auto-oxidation and interaction with singlet oxygen, acids, metals and free radicles (De Moura, Miloff and Boy, 2015). Photo-oxidation of the OFSP puree is usually avoided by storage of the puree away from light. OFSP puree itself has lower levels of β -carotene compared to the tubers due to the processing that entails boiling which is known to result into reduction of all-trans β -carotene by conversion into *13-cis* form as reported by Failla *et al.* (2009). There is also isomerization of β -carotene during storage due to contact with light, heat, acids and oxygen; deteriorating the colour and vitamin A activity (Provesi *et al.*, 2011).

3.5.2 Vitamin A Content of Shelf-Storable OFSP Puree Bread

Shelf storable OFSP puree bread had up to 173.98 μ g RAE/100g in dry weight. This alone has the potential to meet about a half of the recommended dietary intake for children aged 1-3years (300 μ g RAE) (Kurabachew, 2015). The shelf-storable OFSP puree could be a good fortificant for bread up to 3 months at 40% substitution (**Table 3.3**). The β -carotene rich property of OFSP tubers has been exploited through incorporating puree into bread (Sindi *et al.*, 2013). Bread made from four-month old shelf-storable OFSP bread had low β -carotene content. This can be attributed to low levels of β -carotene in the four-month old shelf-storable OFSP puree. β -carotene could not be detected in the wheat bread. The interest was on the crumb as the crust of breads have low levels of β -carotene due to the thermal degradation of the β -carotene during baking (De Moura *et al.*, 2015).

Table 3.2: Beta carotene content of OFSP puree (mg/100g dry weight)

Sample	β-carotene isomer	Period of storage in Months				
		0	1	2	3	4
Fresh puree	13Z	1.34±0.55 ^a	1.57±0.20 ^a	1.57±0.14 ^a	1.57±0.14 ^a	1.41±0.16 ^a
	All <i>E</i>	11.27±1.11 ^a	10.11±1.6 ^{ab}	9.53±0.57 ^b	9.53±0.57 ^b	9.03±0.72 ^{bc}
	9Z	0.15±0.01 ^a	0.11±0.09 ^a	0.15±0.16 ^a	0.15±0.16 ^a	0.05±0.04 ^a
	Total	12.75±1.66 ^a	11.79±1.87 ^a	11.25±0.67 ^b	11.25±0.67 ^b	10.48±0.91 ^{bc}
Treatment 1	13Z	1.34±0.55 ^a	0.93±0.07 ^b	0.71±0.10 ^b	0.58±0.13 ^b	0.75±0.05 ^b
	All <i>E</i>	11.27±1.11 ^a	7.67±0.41 ^b	6.74±0.34 ^{bc}	6.63±0.20 ^{bc}	6.05±0.59 ^c
	9Z	0.15±0.01 ^a	0.22±0.02 ^a	0.16±0.14 ^a	0.06±0.06 ^a	0.12±0.07 ^a
	Total	12.75±1.66 ^a	8.82±0.32 ^b	7.62±0.30 ^{bc}	7.27±0.13 ^c	6.93±0.50 ^c
Treatment 2	13Z	1.34±0.55 ^a	1.53±0.22 ^a	0.86±0.13 ^b	0.71±0.30 ^b	0.71±0.07 ^b
	All <i>E</i>	11.27±1.11 ^a	7.85±1.50 ^b	7.22±0.31 ^c	6.66±0.08 ^c	4.59±0.23 ^d
	9Z	0.15±0.01 ^a	0.40±0.55 ^a	0.18±0.04 ^a	0.18±0.03 ^a	0.14±0.06 ^a
	Total	12.75±1.66 ^a	9.78±0.81 ^b	8.26±0.33 ^c	7.55±0.32 ^c	5.45±0.25 ^d

Values with the same superscript across the row are not significantly different at $P < 0.05$. Treatment 1=0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid, Treatment 2=0.2% potassium sorbate + 0.2% sodium benzoate + 1% citric acid. 13Z=13 Cis β-carotene, All *E*=All Trans β-carotene and 9Z=9 Cis β-carotene.

Table 3.3: Vitamin A (RAE) content of bread made from different treatments of shelf-stable OFSP puree sampled at different storage periods

Sample	Month			
	1	2	3	4
Wheat bread	ND	ND	ND	ND
30% fresh OFSP puree bread	116.86±5.24 ^e	105.37±4.72 ^e	80.47±10.75 ^{bcd}	85.84±11.07 ^{cd}
40% fresh OFSP puree bread	190.65±15.59 ^f	168.31±13.77 ^f	109.93±3.39 ^e	118.73±2.21 ^e
30% SS T1OFSP puree bread	62.66±13.54 ^{bc}	6.58±0.33 ^a	4.63±0.79 ^a	4.94±0.47 ^a
40% SS T1OFSP puree bread	110.71±5.69 ^d	44.05±6.06 ^b	56.40±3.45 ^{bc}	5.23±0.55 ^a
30% SS T2 OFSP puree bread	70.82±10.20 ^{bc}	40.75±6.46 ^b	55.38±1.79 ^b	8.27±1.34 ^a
40% SS T2 OFSP puree bread	83.70±10.54 ^{bcd}	173.98±2.23 ^f	121.30±8.05 ^e	8.66 ±0.55 ^a
LSD 5% (Sample*Month)	30.25			

Values with the same superscript in the table are not significantly different at P< 0.05. 12mg of All trans β-carotene and 24 mg of 13 and 9 cis β-carotene is equivalent to 1000μg RAE (FAO/INFOODS, 2012). SS-shelf-storable, T1 had 0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid and T2 had 0.2% potassium sorbate + 0.2% sodium benzoate + 1% citric acid.

3.5.3 Proximate Composition of Shelf-Storable OFSP Puree Based Bread

The proximate composition of different bread samples produced from shelf-storable puree is shown in **Table 3.4**. There was no significant differences ($P>0.05$) in the proximate composition of breads made from purees sampled at different storage months. This is because vacuum packaging results into insignificant changes in the proximate composition in the purees in storage (Murcia *et al.*, 2003).

The moisture content of OFSP-puree bread was significantly ($p < 0.05$) higher than the wheat bread, ranging from 29.63 to 31.92g 100g⁻¹. There was no significant difference ($P>0.05$) in the moisture content of breads made from fresh puree and that made from shelf-storable OFSP puree. Wheat bread had the lowest moisture content of 27.87 ± 0.48 g 100g⁻¹. Another study by Bonsi *et al.* (2016) found a similar result, OFSP puree based bread had 17 % more moisture content than wheat bread whose value was 26.83 ± 0.94 g 100g⁻¹. This can be attributed to the fact that OFSP puree is known to have higher moisture content than wheat flour, thus bakery products made from the puree have higher moisture content (Ginting and Yulifianti, 2015). Increasing the substitution level of wheat flour with OFSP puree from 30% to 40% amounted to no significant change in the moisture content ($p>0.05$).

Crude ash content of the shelf-storable OFSP puree bread ranged from 2.07 to 2.24g 100g⁻¹ in dry weight which was significantly ($p<0.05$) higher than that of the wheat bread. The flour used in the study had the bran removed during processing. This is known to result into significant loss in the ash content, thus bakery products from such flours have lower ash content (Sibanda *et al.*, 2015). Of all the composite breads, shelf-storable puree-based bread tended to have the highest crude ash content. The crude ash content of shelf-storable OFSP puree bread was not

significantly different ($p>0.05$) from fresh OFSP puree bread. This can be attributed to the use of additional chemical leavening agents, baking soda and baking powder, in both fresh and shelf storable OFSP puree bread; they significantly contribute to the mineral content of bread (Lopes *et al.*, 2017). The sodium content of the bread should be a course for further research for labeling purposes.

3.5.4 Physical Characteristics of Shelf-Storable OFSP Puree Based Bread

There was no significant difference in the loaf weight, loaf volume and specific volume of the breads ($p>0.05$) (**Figures 3.4, 3.5 and 3.6**). The variation in volume, weight and specific volume was not statistically significant ($p>0.05$) considering that enough time was provided for the bread with OFSP puree to proof. Shelf-storable OFSP bread achieved a loaf volume of 2031-2192cm³ and a specific volume of 5.394-5.923cm³/g. This is also attributed to the fact that dough conditioners are used in dough with OFSP puree to improve on the rising of the bread. Dough improvers in form of enzymes improve on dough characteristics such as the texture and fermentation speed which result into improved physical attributes in bread (Lopes *et al.*, 2017). For the shelf-storable OFSP puree, additional chemical leavening agents were used to help overcome the effect of inhibition of the yeast by the preservatives in the puree which are known antifungals (Wen *et al.*, 2016). Chemical leavening agents are known to be fast acting in terms of release of carbon dioxide gas that causes the rising in bread (Lopes *et al.*, 2017). Baking powder and baking soda (sodium bicarbonate) are two chemical leavening agents used in bakery for rising of bakery products.

Table 3.4: Proximate Composition of the OFSP Puree Composite Breads (g 100g⁻¹ dry weight)

Chemical composition	Wheat bread	30% Fresh puree bread	30% T1 SS bread	30% T2 SS bread	40% Fresh puree bread	40% T1 SS bread	40% T2 SS bread
Moisture	27.87±0.48 ^a	30.14±1.69 ^b	32.62±1.95 ^b	29.86±2.91 ^b	31.92±1.66 ^b	29.63±1.07 ^b	31.38±2.89 ^b
Crude ash	1.59±0.49 ^a	1.72±0.26 ^{ab}	1.76±0.24 ^{ab}	2.07±0.13 ^{bc}	2.24±0.26 ^c	2.16±0.37 ^{bc}	2.18±0.24 ^{bc}
Crude fat	6.16±1.05 ^a	5.76±0.18 ^a	5.91±0.50 ^a	5.87±0.42 ^a	4.69±0.90 ^a	5.21±1.07 ^a	6.52±1.81 ^a
Crude protein	11.00±0.81 ^a	11.07±0.51 ^a	10.99±0.15 ^a	10.96±0.58 ^a	11.12±0.93 ^a	10.87±0.28 ^a	10.48±0.22 ^a
Crude fiber	1.23±0.26 ^a	1.83±0.81 ^a	1.95±0.76 ^a	1.39±0.17 ^a	1.49±0.21 ^a	1.46±0.36 ^a	2.24±0.93 ^a
Carbohydrates	79.41±1.13 ^a	78.89±1.32 ^a	78.53±0.42 ^a	78.84±0.88 ^a	79.41±1.14 ^a	79.39±1.84 ^a	77.58±0.73 ^a

Values with the same superscript along a row are not significantly different at P< 0.05. Values expressed in dry weight apart from moisture content, SS (Shelf-storable), T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid.

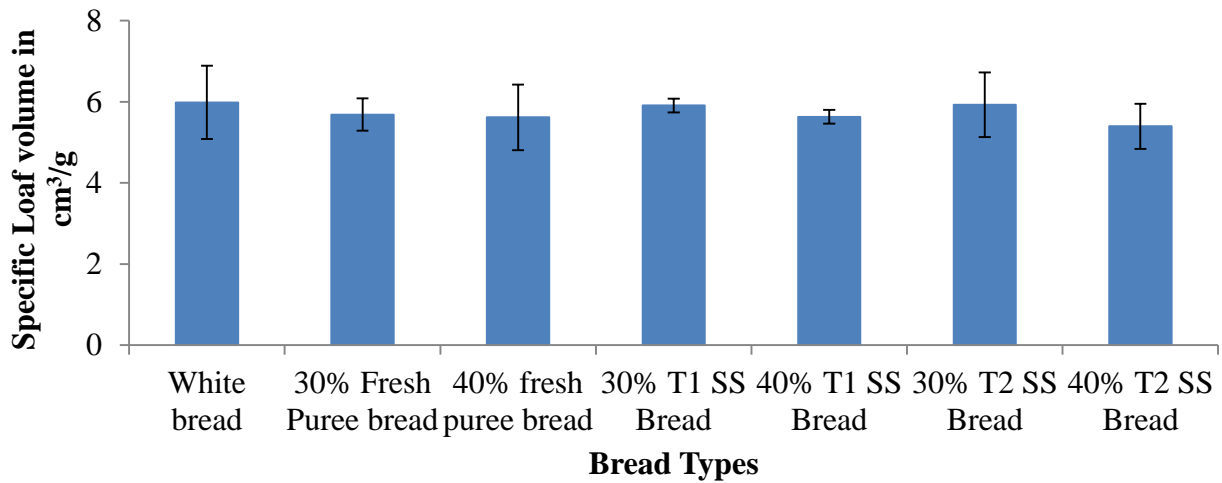


Figure 3.4: Specific Loaf Volume of OFSP puree bread g/cm³. SS-shelf-storable, T1 had 0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid and T2 had 0.2% potassium sorbate + 0.2% sodium benzoate +1% citric acid. The bars indicate the standard error of means.

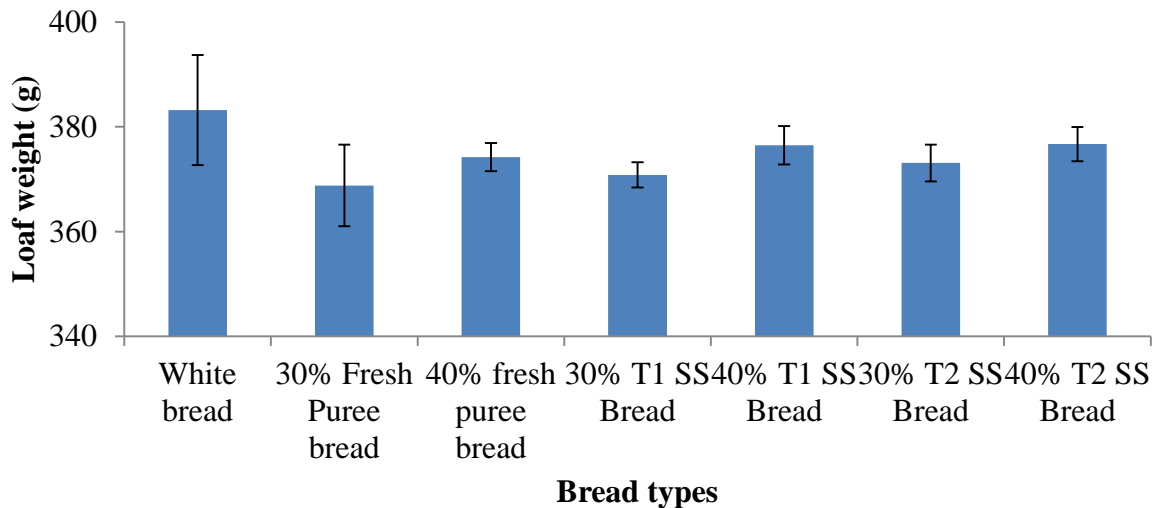


Figure 3.5: Loaf weight of OFSP puree bread in grams. SS-shelf-storable, T1 had 0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid and T2 had 0.2% potassium sorbate + 0.2% sodium benzoate +1% citric acid. The bars indicate the standard error of means.

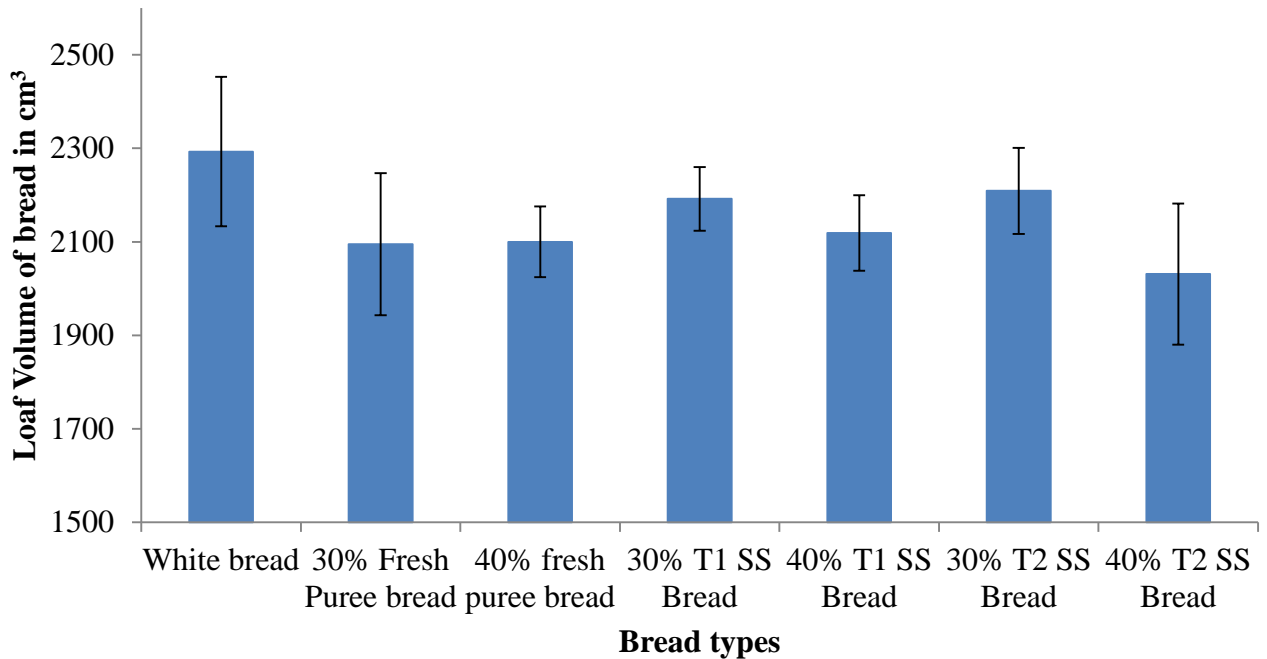


Figure 3.6: Loaf Volume of OFSP puree bread in cm³. SS-shelf-storable, T1 had 0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid and T2 had 0.2% potassium sorbate + 0.2% sodium benzoate +1% citric acid. The bars indicate the standard error of means.

3.6 CONCLUSION

Shelf-storable OFSP puree can be presented as an alternative to fresh OFSP puree in bread baking. Shelf-storable OFSP puree bread had significant levels of β -carotene to act as a fortificant in bread up to a period of three months of its storage. It would thus be possible to exploit shelf-storable bread to expand the production of OFSP puree bread, even scaled down to small-scale producers. The shelf-storable OFSP puree provides crude protein, crude fat, crude fiber and carbohydrate similar to the levels in wheat bread and fresh OFSP puree bread. The incorporation of shelf-storable OFSP into bread has no detrimental effects on the composition of other nutrients in bread.

CHAPTER FOUR: SENSORY ATTRIBUTES AND SHELF-STABILITY OF SHELF-STORABLE ORANGE-FLESHED SWEETPOTATO PUREE-WHEAT COMPOSITE BREAD

4.1 ABSTRACT

The study evaluated the keeping quality and sensory acceptability of bread developed by incorporating shelf-storable OFSP puree as an alternative to fresh puree in the fortification of bread with beta-carotene. There were two treatments for shelf-storable OFSP puree sampled monthly for a period of four months: treatment 1 with 0.5% potassium sorbate, 0.5% sodium benzoate and 1% citric acid and treatment 2 with 0.2% potassium sorbate, 0.2% sodium benzoate and 1% citric acid. Each of these purees was incorporated into bread at 30% and 40% wheat flour substitution. Fresh puree and wheat bread were used as controls. The breads were subjected to descriptive sensory analysis 24 hours after their baking. Microbial plating for yeasts and molds and total aerobic counts were done at days 0, 3 and 7 for samples prepared from stored puree. The results indicate that both the 40% and 30% wheat substitution with either treatment 1 or 2 OFSP puree bread are acceptable to the consumers with overall acceptability of >5 at $p < 0.05$. The saltiness, smoothness and crumb color scores for shelf-storable OFSP puree bread were similar to that of fresh puree bread but significantly ($p < 0.05$) higher than that of wheat bread. Bread in which shelf-storable OFSP puree was incorporated had lower yeast and mold counts than wheat bread ($p < 0.05$). Total aerobic counts in shelf-storable OFSP puree bread prepared from four month old puree had the highest counts as compared to the other months ($p < 0.05$). Shelf-storable OFSP puree bread had similar sensory profile to fresh OFSP puree bread thus can be exploited as an alternative to fresh puree in bread baking. It's recommended to use of shelf-storable OFSP puree to improve the sensory profile of bread.

4.2 INTRODUCTION

Sensory evaluation is the scientific discipline that is employed to measure and interpret consumer responses to the qualities of products based on their perception by their five senses, namely taste, sight, touch, smell and hearing (Sindi *et al.*, 2013). The sensory appeal acts as the first determinant for majority of the consumers' purchase of a food product, thus affect the marketability of a food product (Swahn *et al.*, 2012). Descriptive analysis is used to establish differences in various sensory parameters as influenced by adjustments and changes in the ingredients (Vindras-Fouillet *et al.*, 2014). Descriptive sensory analysis gives the sensory profile of the product; this influences the consumer acceptability of the product.

The incorporation of orange-fleshed sweetpotato (OFSP) puree in bread is aimed at improving the vitamin A status of the consumers as OFSP is known to be rich in beta-carotene, a pro-vitamin A (Low *et al.*, 2007; Bonsi *et al.*, 2014). The use of the OFSP puree over OFSP flour has been shown to be advantageous in terms of economic returns such as reduction in energy consumption and conversion rate; nutritional content such as beta-carotene content; sensory attributes such as color and taste and quality parameters such as loaf volume and texture (Sindi *et al.*, 2013; Muzhingi *et al.*, 2016). Incorporation of any ingredient to improve the nutritional quality of bread must not adversely affect sensory attributes as consumers' perception would define the acceptability of such bread and therefore its marketability. There are indications that OFSP puree bread has a higher acceptability and consumers are willing to pay more for it compared to the wheat wheat bread (Wambui, 2017).

Currently OFSP fresh puree is used for bakery application in Kenya, and it is delivered to end users in a frozen state. In order to reduce the cost of cold chain transportation, manage

seasonality of OFSP fresh root supply for puree production and expand on the user base of OFSP puree, International Potato Center sub-Saharan Africa (CIP-SSA) embarked on a project to develop a shelf-storable OFSP puree which will make bakery products similar to those from frozen OFSP puree (Bocher *et al.*, 2017). Shelf-storable OFSP puree can be made using aseptic processing and packaging technologies. However, in Kenya, it is most cost effective and easier to use chemical preservatives such as benzoate, sorbate and citric acid with Modified Atmosphere packaging to make OFSP puree shelf-stable. However, it is currently not known whether products made from shelf-storable OFSP puree are similar to those made from fresh OFSP puree in terms of taste, color, flavor and shelf-stability. This study therefore sought to establish the sensory profile and microbial quality of bread developed from shelf-storable OFSP puree.

4.3 MATERIALS AND METHODS

4.3.1 Sample Preparation

Bread samples were prepared as per the methods described in **section 3.2.1**.

4.4 ANALYTICAL METHODS

4.4.1 Determination of pH

The pH of the puree and dough was determined using a pH meter (Mettler Toledo, USA). A sample of 10 g was homogenized in 20 ml of deionized water and the pH determined using a calibrated pH meter.

4.4.2 Sensory Analysis

Descriptive sensory analysis of the breads was done in Homabay County using a semi-trained panel. A panel of thirty people with an attrition of 10% (16 males and 14 females aged between 20 and 55 years) was randomly selected among locals who were regular consumers of bread in Homa Bay County. Verbal consent of the panelist was sought with the study being explained to the panelists. A 30 minute training session was held for the panelists on predetermined descriptors of the bread samples as established by Al-Saleh and Brennan (2012) and Vindras-Fouillet *et al.* (2014), namely appearance (crumb and crust color), odor (yeasty and grainy), taste (saltiness, sweetness and sourness), texture (crispiness and smoothness), long lasting taste and overall acceptability (**Appendix 1 and 2**). The panel was used for the entire period of study of four months.

The bread samples were marked using randomly chosen three digit numbers in duplicates and presented to the panel to assess. The intensity of each attribute was scored using a 9-point verbally anchored scale according to procedures established by Nordic Committee on Food Analysis (Nordic Committee on Food Analysis, 2015). The samples were scored from 1-extremely low to 9-extremely high. The panelists were provided with water to refresh their palate before evaluating successive samples.

4.4.3 Microbial Analysis

Bread samples prepared as explained in **Section 3.2.1.** at the University of Nairobi Pilot Plant were taken to Department of Food Science, Nutrition and Technology Food Microbiology Laboratory for determination of microbial shelf-stability. The breads were stored at ambient temperatures (15-25°C) and sampling for microbial analysis done at day 0, 3 and 7.

4.4.3.1 Total viable count

The total viable count was done as per method 42-11-01 of AACC (2000). A bread sample of 25g will be put into 225 ml of 0.85% sodium chloride diluent. Serial dilutions of the samples were prepared. Molten plate count agar media was prepared according to the Manufacturer's directions. Pour plating technique was used in plating 1ml of each dilution in triplicate. The plates were incubated upside down at 30°C for 72 hours and enumerated using colony counter technique and expressed as log cfu g⁻¹.

4.4.3.2 Yeast and moulds

The yeast and mould counts was done as per method 42-50-01 of AACC (2000). A 25 g bread sample was transferred into 225 mL of 0.85% sodium chloride diluent and mixed for 30 seconds. Serial dilution of the sample was prepared up to 10⁻⁶. Molten potato dextrose agar media was prepared as per the Manufacturer's directions. A Pasteur pipette was used to transfer 1ml of each serial dilution into a sterile petri-dish under aseptic condition. Pour plating of the dilutions was done in triplicate for each dilution. The plates were incubated upside down at 30°C for 72 hours, then enumerated using colony count technique with microbial counts expressed in log cfu g⁻¹.

4.4.4 Statistical Analysis

The data were analyzed in Genstat version 15. Descriptive statistics such as the mean of replicates and standard deviation of the means of sensory scores and microbial counts were obtained. ANOVA test was used to test the significant differences of pH and microbial counts in randomized block design while the sensory scores were in split-plot design. Fischer's LSD test was used to separate means that were significantly different.

4.5 RESULTS AND DISCUSSION

4.5.1 pH of Dough

The pH of treatment 1 and treatment 2 shelf-storable purees were found to be 4.30 and 4.63, respectively. All the dough had a slightly acidic pH which is attributed to the leavening activity by the yeast in the dough, this is necessary for the flavor of the breads (Struyf *et al.*, 2017). The pH of the dough made by incorporating shelf-storable OFSP puree was adjusted to between 5.513 and 5.876 which were significantly ($p < 0.05$) higher than the dough for wheat bread (Figure 4.1). This can be attributed to the basic nature of leavening agents which is known to raise pH of products (Tejinder *et al.*, 2015).

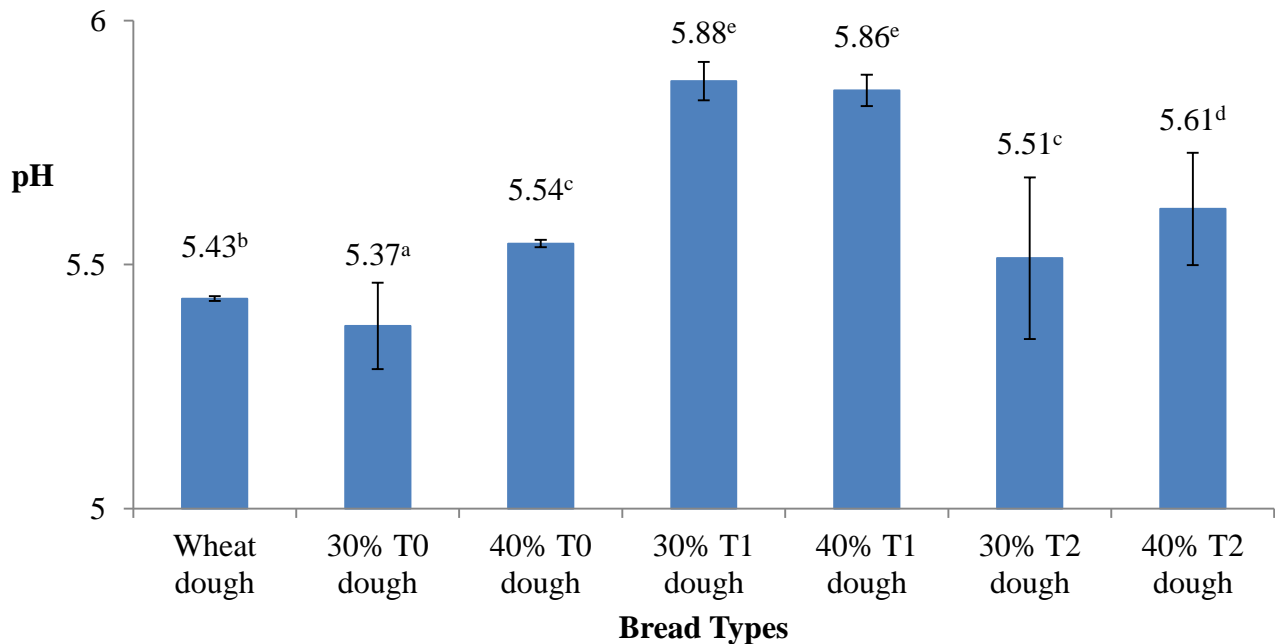


Figure 4.1: pH of dough used in bread baking. Different letters are significantly different at $P < 0.05$. T0= frozen puree, T1= puree treated with 0.5% potassium sorbate + 0.5% sodium benzoate + 1% citric acid and T2= puree treated with 0.2% potassium sorbate + 0.2% sodium benzoate + 1% citric acid. The bars indicate the standard error of means.

4.5.2 Sensory Attributes of OFSP and Wheat Bread

Significant differences ($p < 0.05$) existed in the crust color, crumb color, saltiness, smoothness and overall acceptability (**Table 4.1**). The sensory perception of crust color and smoothness significantly varied among breads developed from shelf-storable OFSP puree of different storage periods as shown in **Tables 4.2 and 4.3**.

Both the fresh and shelf-storable OFSP puree of both treatments had golden yellow crumb color (sensory score > 5). The consumer rating of the crumb color is in agreement with studies done by Bonsi *et al.* (2014), where the distinctive color of bread developed from OFSP puree was noted across varied study populations in Ghana. OFSP puree imparts golden yellow color to bread that is highly likable to consumers, thus presents an economic advantage (Low and Jaarsveld, 2008). OFSP puree bread is known for its distinctive golden yellow color in the Kenyan markets (Wambui, 2017). The golden yellow color is attributed to beta-carotene which on top of the nutritional advantages it presents, can also serve as a natural food colorant. With most consumers not liking artificial colorants, the food coloring property of beta-carotene is commendable in bread.

OFSP bread with 40% fresh puree incorporated was more acceptable of the breads ($p < 0.05$) while the 30% fresh OFSP puree bread had similar acceptability scores to both treatment 1 and 2 shelf-storable OFSP puree bread at 40% level of substitution and wheat bread ($P > 0.05$). This finding was different from what was reported by Sindi *et al.* (2013) found, where bread with 30% incorporation of fresh OFSP puree was more acceptable than wheat bread. Another study by Awuni *et al.* (2017) that focused on bread with 46% fresh puree incorporated, attributed the higher acceptability scores of fresh OFSP bread over wheat bread to its golden yellow color.

Generally, the shelf-storable OFSP puree breads had acceptability scores of 5.14 and 5.78, $p < 0.05$. An overall acceptability score of ≥ 5 for OFSP bread on a 9-point hedonic rating is indicative of consumer liking of the product (Bonsi *et al.*, 2014). With evidence of a higher willingness to pay by consumers as shown by consumer profile studies in Kenya (Wambui, 2017), shelf-storable OFSP puree bread can serve to expand the accessibility of this bread.

There were significantly ($p < 0.05$) higher saltiness scores in all shelf-storable OFSP puree breads as compared to normal wheat bread (3.99). Both treatments of shelf-storable OFSP puree breads achieved similar saltiness scores to fresh puree breads ($p > 0.05$). Consumers rated saltiness of bread developed from both treatments of shelf-storable purees as neither too high nor too low. Saltiness can greatly impact on the acceptability of these breads as taste has been established as an influencing factor on consumer preference of bread (López *et al.*, 2013). The firmness and porosity of the bread crumbs is known to influence the release of sodium ions during mastication; softer breads such as the OFSP puree bread will have a higher saltiness (Pflaum *et al.*, 2013). The role of the porosity and firmness of the OFSP puree bread crumbs should be evaluated to provide further evidence of the influence of the porosity and distribution of gas cells on saltiness in OFSP puree bread with prospects of reducing salt levels used. The levels of sodium ions in the bread were not checked but this would need to be probed further as higher sodium levels would also prove unhealthy (Belz *et al.*, 2012).

Incorporation of OFSP puree resulted into increased smoothness of the bread crumb as perceived by the panelists, $p < 0.05$. Bread with 40% OFSP puree incorporated had smoother crumbs than those with 30% of OFSP puree incorporated as shown in **Table 4.3** ($p < 0.05$). Both treatments of shelf-storable OFSP puree achieved breads with greater smoothness (ranging from 4.82 ± 0.97 to

6.53 ± 0.26) than the control wheat bread (3.16 ± 0.0), p<0.05. Bread with 40% treatment 1 shelf-storable OFSP puree incorporated was perceived to be the smoothest (6.53±0.26), p<0.05. Wheat bread had the least average sensory scores for the smoothness of the crumb, 3.99 ± 0.04. Significant differences (p<0.05) existed in breads sampled at different months but with maintained trend of higher scores for OFSP puree bread than for wheat bread. A study done among Kenyan consumers, gave similar results showing higher likability of OFSP bread by consumers because of its soft crumb (Wambui, 2017). Smoothness is an important attribute in bread as it has also been established that consumers believe smooth breads are fresh (Heenan *et al.*, 2009). Consumers would therefore prefer breads with either shelf-storable or fresh OFSP puree incorporated due to their soft crumb texture.

Both treatments of OFSP purees at either level of substitution, 30% or 40%, had an intensively browner color as compared to the wheat bread. There were no significant differences (P>0.05) between the crust color of 30% OFSP puree bread and 40% OFSP puree bread for the respective purees. The crust color was significantly different (p<0.05) for breads sampled at different months but with a maintained trend of lower scores for the control wheat breads, 3.13 ± 0.72 to 3.83 ± 0.09 (p<0.05), as shown in **Table 4.3**. The browning on the crust of bread is usually due to non-enzymic chemical reactions involving sugars (caramelization) as well as sugars and amino acids (maillard reaction) (Purlis, 2010). The intensity of the crust color and the thickness of the crust in bread have been associated with moisture content and water activity when temperature as a factor has been standardized. Intensity of browning and size of the crust increases as more water vapor is lost through vapor pressure gradient (Chhanwal and

Anandharamakrishnan, 2014); higher moisture content as in the case of OFSP puree based bread would result into a thicker and more browner crust.

Table 4.1: Sensory scores of shelf-storable OFSP puree-wheat composite bread

Parameters	Samples						
	Wheat bread	30% Fresh puree bread	40% Fresh puree bread	30% T1 SS bread	40% T1 SS bread	30% T2 SS bread	40% T2 SS bread
Crispiness	3.76±1.90 ^a	4.29±2.10 ^a	4.44±2.03 ^a	4.28±1.94 ^a	4.36±1.86 ^a	4.22±2.06 ^a	4.21±2.10 ^a
Crust color	3.19±1.36 ^a	5.83±1.34 ^{cd}	6.16±1.83 ^d	5.74±1.63 ^{bc}	5.92±1.53 ^{cd}	5.45±1.49 ^b	5.78±1.55 ^{bc}
Crumb color	2.74±0.95 ^a	6.20±1.02 ^d	6.79±1.22 ^e	5.83±1.43 ^c	6.05±1.43 ^c	5.29±1.33 ^b	6.03±1.50 ^c
Grainy odor	4.82±1.78 ^a	4.64±1.85 ^a	4.47±1.91 ^a	4.72±1.92 ^a	4.36±1.92 ^a	4.28±1.89 ^a	4.33±1.85 ^a
Yeasty odor	3.79±0.78 ^a	4.77±0.97 ^a	4.83±0.06 ^a	4.73±0.16 ^a	4.28±0.20 ^a	4.38±1.04 ^a	4.21±1.02 ^a
Saltiness	3.99±0.04 ^a	4.61±0.85 ^b	4.67±0.86 ^b	4.63±0.04 ^b	4.57±1.97 ^b	4.62±1.00 ^b	4.72±0.13 ^b
Smoothness	3.16±0.27 ^a	5.36±0.11 ^c	5.11±0.94 ^{bc}	5.44±0.27 ^c	6.53±0.26 ^d	4.82±0.97 ^b	5.13±0.96 ^{bc}
Sourness	4.19±1.06 ^a	3.98±1.91 ^a	4.21±1.98 ^a	4.11±1.88 ^a	3.97±1.99 ^a	4.07±1.98 ^a	4.29±1.21 ^a
Sweetness	4.55±1.98 ^a	5.01±1.92 ^a	4.80±1.93 ^a	4.31±1.85 ^a	4.13±1.94 ^a	4.17±1.05 ^a	4.44±1.85 ^a

Table 2: Cont.

Parameters	Samples						
	Wheat bread	30% Fresh puree bread	40% Fresh puree bread	30% T1 SS bread	40% T1 SS bread	30% T2 SS bread	40% T2 SS bread
Long lasting taste	4.90±1.96 ^a	4.70±2.04 ^a	4.23±1.92 ^a	4.35±2.12 ^a	4.42±2.07 ^a	5.16±2.31 ^a	4.83±2.21 ^a
Overall Acceptability	6.01±2.06 ^b	6.23±1.94 ^b	6.70±1.91 ^c	5.14±1.46 ^a	5.78±1.73 ^b	5.29±2.37 ^a	5.74±1.93 ^{ab}

Means with the same superscript across a row are not significantly different ($p>0.05$). SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid

Table 4.2: Sensory Scores for crust color of bread made from shelf-storable OFSP puree and wheat

Months	Sample						
	Wheat bread	30% Fresh puree bread	40% Fresh puree bread	30% T1 SS bread	40% T1 SS bread	30% T2 SS bread	40% T2 SS bread
1	3.63±0.45 ^b	5.90±0.06 ^{de}	5.70±0.96 ^{cde}	4.97±0.24 ^c	5.50±0.33 ^{cd}	4.97±0.47 ^c	5.46±0.55 ^c
2	2.73±0.25 ^a	5.93±0.46 ^{de}	6.17±0.93 ^{de}	6.40±0.89 ^e	6.17±0.84 ^{de}	5.50±0.59 ^{cd}	6.10±0.42 ^{de}
2	2.70±0.34 ^a	5.53±0.22 ^{cd}	6.00±0.83 ^{de}	5.77±0.43 ^{de}	5.97±0.65 ^{de}	5.60±0.20 ^c	5.57±0.62 ^{cd}
4	3.70±0.09 ^b	5.93±0.57 ^{de}	6.77±0.46 ^{ef}	5.83±0.62 ^{de}	6.03±0.22 ^{de}	5.73±0.57 ^{de}	5.97±0.61 ^{de}
LSD	0.74						
(5%)							

Means with the same superscript are not significantly different ($P>0.05$). SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid

Table 4.3: Sensory scores for smoothness of bread made from shelf-storable OFSP puree and wheat flour

Months	Sample						
	Wheat bread	30% Fresh puree bread	40% Fresh puree bread	30% T1 SS bread	40% T1 SS bread	30% T2 SS bread	40% T2 SS bread
1	3.47±0.01 ^a	4.83±1.01 ^b	4.83±0.70 ^b	4.90±0.88 ^{bc}	6.30±0.02 ^{ef}	3.70±0.47 ^a	5.46±0.76 ^{bcd}
2	3.83±0.09 ^a	5.90±1.26 ^{def}	5.07±0.98 ^{bc}	5.67±0.27 ^{cde}	6.50±0.31 ^{ef}	5.03±0.11 ^{bc}	6.10±0.90 ^{def}
2	3.13±0.72 ^a	5.37±0.85 ^{bcd}	5.00±1.21 ^{bc}	5.93±0.48 ^{def}	6.73±0.51 ^f	3.13±1.20 ^a	5.57±1.10 ^{bcd}
4	3.20±0.16 ^a	5.33±1.26 ^{bc}	5.53±0.83 ^{bcd}	5.27±0.17 ^{bcd}	6.60±0.16 ^f	3.20±0.66 ^a	5.97±0.63 ^{def}
LSD	0.83						
(5%)							

Means with the same superscript are not significantly different ($P>0.05$). SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid

4.5.3 Microbial Quality

The microbial load of the bread samples on different days are as shown in **Tables 4.4 and 4.5**. The wheat bread had higher yeast and mold counts after 7 days of storage across all months, averaging $4.70 \pm 0.91 \log \text{ cfu g}^{-1}$ ($p < 0.05$). Both treatment 1 and treatment 2 shelf-storable OFSP puree breads showed no visible yeast and mould growths up to seven days of storage whereas there was visible yeast and mould growths after three days of storage for wheat wheat bread. Studies have shown that OFSP puree breads have low water activity than wheat bread, thus lower yeast and molds growth (Wambui, 2017). There was no growth for yeast and mould counts for 0 and 3 days old breads that had been developed from both fresh and shelf-storable OFSP purees sampled at different months. The low counts in the first three days in the breads is attributed to baking that is known to results in destruction of most microorganisms (Smith *et al.*, 2004).

The baseline analysis of the breads yielded no growth for total aerobic counts for breads developed from treatment 1 and 2 shelf-storable OFSP puree for as freshly baked breads are free of bacterial contamination (Smith *et al.*, 2004). Microbial growths were noted for aerobic plate count on shelf-storable OFSP puree bread at three and seven days of storage with increasing counts as the period of storage of the shelf-storable OFSP puree increased ($p < 0.05$). This can be attributed to the continued microbial growth in chemically preserved puree (Hashmi *et al.*, 2007), some of which produce spores that can withstand baking temperatures. All the breads from three month old puree, except for the breads with fresh OFSP puree incorporated, at 7 days of storage had more than $6 \log \text{ cfu g}^{-1}$ ($p < 0.05$) thus unacceptable for consumption as per the International

Commission for Microbiological Specification that has set a microbial load limit of 10^6 cfu g⁻¹ for ready-to-eat foods like bread (Demilew *et al.*, 2017).

Table 4.4: Yeast and Mould Counts of OFSP–wheat composite breads (log cfu g-1)

Days bread was stored	Period of storage of puree (Months)	Bread samples							
		Wheat	30% fresh OFSP puree	40% fresh OFSP puree	30% SS T1 OFSP puree	40% SS T1 OFSP puree	30% SS T2 OFSP puree	40% SS T2 OFSP puree	
0	1	ND	ND	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND
	4	ND	ND	ND	ND	ND	ND	ND	ND
3	1	ND	ND	ND	ND	ND	ND	ND	ND
	2	2.45±0.45	ND	ND	ND	ND	ND	ND	ND
	3	1.94±0.05	ND	ND	ND	ND	ND	ND	ND
	4	1.96±0.05	ND	ND	ND	ND	ND	ND	ND

Values with similar lowercase letters in the superscripts along a column and uppercase letters along a row are not statistically different ($p>0.05$). *ND-not detected, SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid.

Table 4.4: Cont.

Days bread was stored	Period of storage puree (Months)	Breads						
		Wheat	30% fresh	40% fresh	30% SS T1	40% SS T1	30% SS T2	40% SS T2
		OFSP puree	OFSP puree	OFSP puree	OFSP puree	OFSP puree	OFSP puree	OFSP puree
7	1	5.19±0.15 ^a	3.99±0.03 ^a	3.75±0.13 ^a	3.62±0.12 ^a	3.49±0.21 ^a	3.31±0.05 ^a	2.69±0.54 ^a
	2	5.15±0.20 ^a	4.19±0.13 ^a	3.76±0.23 ^a	3.61±0.07 ^a	3.38±0.06 ^a	3.40±0.09 ^a	2.86±0.35 ^a
	3	3.76±1.13 ^a	3.43±1.36 ^a	3.76±1.23 ^a	3.41±0.77 ^a	3.89±0.13 ^a	3.84±0.27 ^a	3.40±0.55 ^a
	4	4.70±1.16 ^a	3.87±1.06 ^a	3.76±1.03 ^a	3.79±0.07 ^a	3.60±0.13 ^a	3.53±0.17 ^a	2.97±0.22 ^a
	Average	4.70±0.91 ^C	3.87±0.76 ^B	3.76±0.62 ^B	3.55±0.40 ^B	3.59±0.26 ^B	3.52±0.28 ^B	2.98±0.53 ^A

Values with similar lowercase letters in the superscripts along a column and uppercase letters along a row are not statistically different ($p>0.05$). *ND-not detected, SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid.

Table 4.5: Total Aerobic Counts for OFSP-wheat composite breads (log cfu g-1)

Days bread was stored	Period of storage puree (Months)	Bread							
		Wheat	30% fresh OFSP puree	40% fresh OFSP puree	30% SS T1 OFSP puree	40% SS T1 OFSP puree	30% SS T2 OFSP puree	40% SS T2 OFSP puree	
0	1	ND	ND	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND
3	1	4.11±0.02 ^a	1.66±0.42	1.70±0.07	1.75±0.19 ^a	1.78±0.25 ^a	1.67±0.06 ^a	1.64±0.16 ^a	
	2	4.33±0.04 ^a	ND	ND	1.65±0.01 ^a	1.96±0.00 ^a	1.81±0.23 ^a	1.83±0.25 ^a	
	3	5.46±0.01 ^b	ND	ND	4.11±0.02 ^b	4.27±0.02 ^b	3.13±0.01 ^b	3.52±0.04 ^b	
	4	4.62±0.11 ^b	ND	ND	2.93±0.08 ^b	5.35±0.03 ^b	4.63±0.09 ^b	5.45±0.04 ^b	
	Average	4.63±0.63 ^E	1.66±0.42 ^A	1.70±0.07 ^A	2.61±1.25 ^B	3.34±1.89 ^D	2.81±1.51 ^{BC}	3.11±1.94 ^{CD}	

Values with similar lowercase letters in the superscripts along a column and uppercase letters along a row are not statistically different ($p>0.05$). *ND-not detected, SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid.

Table 4.5: Cont.

Days bread was stored	Period of storage of puree (Months)	Breads						
		Wheat	30% fresh	40% fresh	30% SS T1	40% SS T1	30% SS T2	40% SS T2
		OFSP puree	OFSP puree	OFSP puree	OFSP puree	OFSP puree	OFSP puree	OFSP puree
7	1	4.76±0.75 ^a	4.12±0.99 ^a	4.00±0.87 ^a	3.48±0.03 ^a	3.57±0.10 ^a	3.67±0.08 ^a	3.81±0.14 ^a
	2	5.14±0.11 ^a	3.59±0.23 ^a	3.50±0.01 ^a	4.70±0.17 ^b	4.76±0.03 ^b	4.75±0.51 ^b	5.01±0.13 ^b
	3	5.40±0.31 ^a	3.53±0.04 ^a	3.76±0.11 ^a	5.15±0.02 ^c	5.54±0.23 ^c	4.80±0.21 ^b	5.01±0.07 ^b
	4	6.14±0.11 ^b	3.76±0.02 ^a	3.82±0.05 ^a	7.28±0.07 ^d	8.33±0.06 ^d	5.86±0.04 ^c	6.21±0.07 ^c
	Average	5.36±0.73 ^{CD}	3.75±0.58 ^A	3.77±0.49 ^A	5.15±1.68 ^{BC}	5.55±2.15 ^D	4.77±0.96 ^B	5.01±1.04 ^B

Values with similar lowercase letters in the superscripts along a column and uppercase letters along a row are not statistically different ($p>0.05$). *ND-not detected, SS-shelf-storable, T1 had 0.5% potassium sorbate+0.5% sodium benzoate+1% citric acid and T2 had 0.2% potassium sorbate+0.2% sodium benzoate+1% citric acid.

4.6 CONCLUSION

Substitution of wheat flour with 30% and 40% of different treatments of shelf-storable OFSP puree achieves similar sensory parameters to fresh puree based bread. Bread in which 40% fresh OFSP puree is incorporated has a higher acceptability than shelf-storable OFSP puree based bread. Prolonged storage of shelf-storable OFSP puree increases the aerobic counts in the bread but the yeast and molds are low. The study established that shelf-storable OFSP puree can be used as an alternative to fresh OFSP puree in bread baking with minimal alterations to the sensory quality and shelf-stability.

CHAPTER FIVE: GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 GENERAL CONCLUSIONS

The period of storage of the shelf-storable OFSP puree affects the beta-carotene content of the breads; shelf-storable OFSP puree is a good fortificant of bread up to three months of its storage. Incorporation of shelf-storable OFSP puree into bread increases the moisture and crude ash contents but has no effect on the loaf weight, volume and specific volume of the breads.

Notwithstanding the period of storage and level of preservative used, shelf-storable OFSP puree when incorporated into bread gives a product that is acceptable to the consumers. It is also noted that incorporation of shelf-storable OFSP puree into bread gives the product a sensory profile similar to fresh OFSP puree based bread, save for the crumb and crust colors and smoothness. The period of storage is of insignificant impact on the sensory profile.

Incorporation of shelf-storable OFSP puree into bread has a similar effect to fresh OFSP puree in terms of limiting growth of yeasts and moulds thus microbial shelf-life of the product is increased. The use of shelf-storable OFSP puree as an alternative to fresh puree would have less undoing to the bread quality but with limited microbial shelf-life.

5.2 GENERAL RECOMMENDATIONS

The use of shelf-storable OFSP puree as a cost-effective and convenient alternative functional ingredient to the capital-intensive and much expensive frozen puree in bread production is recommended. Adoption of the shelf-storable OFSP puree is much more beneficial to the small-scale bakeries that lack sufficient capital and finances to be involved in the cold chain supply. It

is essential for further work to ensure minimal nutritional changes in the puree to ensure its use as a fortificant in bread can be maximized. The porosity and smoothness of crumbs of OFSP puree based bread also presents an opportunity for possible reduction of the salt levels used in bread production.

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APPENDICES

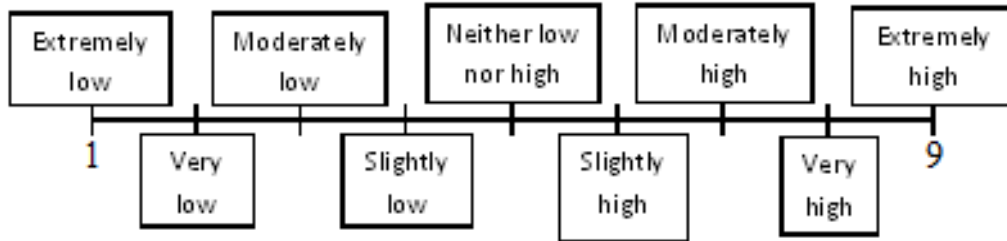
APPENDIX 1: BASELINE SENSORY EVALUATION QUESTIONNAIRE

Date..... Gender..... Age.....yrs

Respondent number.....

Kindly rate the intensity of the sensory attributes from 1 to 9s

Parameters	Samples						
	A	B	C	D	E	F	G
Crust color							
Crumb color							
Yeasty odor							
Grainy odor							
Sweetness							
Saltiness							
Smoothness							
Crispiness							
Long lasting taste							
Overall Acceptability							



THANK YOU FOR PARTICIPATING

APPENDIX 2: TRAINING MANUAL

Explanation of the sensory attributes in the questionnaire

Sensory attribute	Description
<i>Appearance</i>	
Intensity of crust colour	The intensity of perceived brown colour of the crust (from 1- extremely low to 9-extremely high)
Intensity of crumb colour	The intensity of perceived colour darkness of the crumb ranging from white to yellow (from 1- extremely low to 9-extremely high)
<i>Odour</i>	
Yeasty odour	The intensity of the odour due to aromatic exchange resulting from yeast fermentation (from 1- extremely low to 9-extremely high)
Grainy odour	The intensity of the odour from aromatic impression of cereal derived products (from 1- extremely low to 9-extremely high)
<i>Taste</i>	
Sweetness	The intensity of the sugariness of the product of which sucrose is typical (from 1- extremely low to 9-extremely high)
Sourness	The intensity of the taste associated with acids (from 1- extremely low to 9-extremely high)
Saltiness	The intensity of the taste elicited by sodium chloride (from 1- extremely low to 9-extremely high)
<i>Texture</i>	
Crispiness	The intensity of perceived crispiness of the sample crust (from 1- extremely low to 9-extremely high)
Smoothness	The intensity of perceived smoothness of the slices as perceived by the lips (from 1- extremely low to 9-extremely high)
Long lasting taste	Period it takes the aroma of the bread to clear from the mouth (from 1- extremely short to 9- extremely long).
Overall acceptability	The overall liking of the sample by the panelist (1-Dislike extremely to 9-like extremely)
